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MOLYBDENUM METABOLISM AND INTERACTIONS IN RUMINANTS AND MONOGASTRIC ANIMALS

By

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INTRODUCTION

The metabolism of molybdenum and its interactions with other minerals has received considerable attention since this mineral was first associated with an apparent copper deficiency in cattle grazing on pastures containing normally adequate levels of copper (38). Subsequent investigations have shown that molybdenum tends to increase the copper requirements and that copper deficiency resulting from a low level of copper in the forage or the presence of higher than normal levels of molybdenum may be alleviated by the administration of copper (39, 40). Apparently molybdenum is not the only "conditioning factor" in copper deficiency. Hypocuprasmia has been observed in cattle on pastures with normal copper and molybdenum levels (1, 2, 13, 57) and in one case copper therapy did not elicit a satisfactory response, however, there was a rapid response when copper and cobalt were administered together (13). A "conditioned copper deficiency" in rats resulting from the feeding of molybdenum was found to respond to both copper and whole liver therapy (79). The copper content of the liver was not sufficiently high to account for the therapeutic action. Molybdenum toxicity studies with young chicks revealed that copper had only a slight therapeutic effect for this species (63). The occurrence of a natural substance believed to be organic in nature and capable of reducing blood molybdenum and maintaining normal copper levels in molybdenum and copper supplemented sheep has been demonstrated (30). The distribution of such a substance could account for many of the divergent effects and species

variations observed in copper-molybdenum studies.

The purpose of this investigation was to provide additional basic information on molybdenum-copper, calcium and phosphorus interactions and to determine the effect of molybdenum on "D" vitamin utilization in cattle and rats. In carrying out the investigation the effects of high dietary levels of molybdenum on growth, calcium, phosphorus and molybdenum utilization and adrenal function were studied.

The investigation was facilitated by the use of radioactive isotopes of calcium and molybdenum. Chemical analyses were made for copper, calcium and phosphorus. "D" vitamins were assayed for by microbiological methods. Adrenalectomies were performed on both weanling and adult rats.

LITERATURE REVIEW

The physiological role of molybdenum in animal nutrition has not yet been established and it is generally regarded as an adventitious rather than an essential element. This mineral is widespread in the animal kingdom (76). Studies to determine whether it was an essential element for animals were undertaken by Teresi et al. (111) who concluded that if molybdenum was required by the rat, that it was at a concentration of less than five parts per million (p.p.m.). The essential nature of molybdenum for certain micro-organisms and higher plants is well established and has been the subject of recent reviews (9, 44, 73, 104, 116, 117).

Molybdenum and Ruminant Nutrition: Molybdenum is one of the more recent additions to the list of minerals associated with debilitating deficiency diseases of nutritional origin. Ferguson et al. (38) in investigating the causes of "teartness", a condition of cattle characterized by severe and persistent scouring and loss of condition, found that the molybdenum content of "teart" pastures was considerably above normal and ranged from twenty to 100 p.p.m. in the dry matter. In subsequent investigations (39) they demonstrated that healthy pastures could be made "teart" by top dressing with sodium molybdate and the condition could also be produced by feeding sodium molybdate. Molybdenum was thus implicated as a factor in both natural and induced "teartness". Drenching or feeding cows with two grams of copper sulfate and young stock with one gram per head daily cured or prevented scouring

on the very "teart" land. Ferguson et al. (10) in a later report confirmed earlier work and indicated that although the scouring encountered on "teart" pastures could be prevented by the administration of copper sulfate, the affected animals did not exhibit signs of copper deficiency.

Britton and Goss (14) investigated a cattle disease, with similar symptoms, that had long been endemic in the southwestern part of the San Joaquin Valley in central California. The affected cattle became emaciated, showed an intense liquid diarrhea full of gas bubbles and on young heifers the vulva became swollen. Depigmentation occurred in both dairy and beef animals and anemia was marked. In this area young cattle were more susceptible than older ones and dairy cattle were more susceptible than beef animals. Sheep were rarely affected and horses and swine were reported resistant. Symptoms appeared from one to seven months after cattle were placed on affected pastures. Alfalfa samples from this area had a molybdenum content of from six to thirty six p.p.m. Barshad (8) in a later study of the condition in this area reported that in severe cases the hair sheds in spots leaving reddened, dry and cracked skin. Young animals also showed a stiffness in the hind legs. Davis (28) has observed similar conditions in the Everglades region of Florida where soils are low in copper and have moderate levels of molybdenum. The molybdenum content of the forage in this area averages between eleven and sixteen p.p.m. Experimental work has demonstrated that cattle, in this area, have an increased need for copper, and without supplementation there is a disturbance in the metabolism of the bone that results in increased fragility and susceptibility to fractures.

The bone changes are apparently associated with phosphorus metabolism.

Cunningham (24, 26) has described a condition in cattle, referred to as "peat scours", which is very similar to that observed in cattle on the muck soils in Florida (28). The most characteristic symptom of "peat scours" is the persistent severe debilitating scouring. Scouring was observed to occur whenever there was a flush growth of pasture. Spring scours persist for several months and are usually most marked after calving. Calves do poorly, breeding is impaired and there is usually some degree of anemia. Scouring may occur within two to three weeks after animals are placed on spring grass but symptoms do not reach maximum severity until after grazing for a year or more in the affected area. On some areas, marked bone fragility occurs in calves from the age of about three months onward. The anemia observed in affected animals is interesting in that it is seasonal and parallels the incidence of scouring. "Peat scours" pastures are similar to the muck land pastures of Florida in that they are moderately deficient in copper and have a molybdenum content ranging from 2.5 to eleven p.p.m. Cunningham (26) has established a relationship between the level of molybdenum in pastures and the occurrence of scours in affected areas. The molybdenum content of pastures was found to be directly related to rainfall and the stage of growth. Barshad (8) has observed that young plants grown in affected areas were more injurious to cattle than were mature plants even though the latter were higher in total molybdenum. These observations are particularly interesting since the seasonal outbreaks of cobalt deficiency on cobalt deficient terrain are generally preceeded by a marked fall in the cobalt content of pastures and this

is frequently the consequence of a rapid growth of grass (71). The inverse relationship between cobalt and molybdenum content of forage during periods of rapid growth would be of considerable importance if they have an interrelationship in animal nutrition. Milliken (77) has demonstrated that molybdenum will relieve cobalt toxicity in certain plants. The mechanism here is unknown but it might be through a complexing action.

The interrelationships between copper and molybdenum have been widely investigated. Dick and Bull (29) have observed that an increase in the molybdenum intake of cattle and sheep resulted in lowered storage of copper in the liver. Cesar et al. (21) have reported similar findings in cattle and have indicated that these conditions occur naturally in the Everglades region of Florida. Davis (26) in reviewing their studies on interrelationships of copper, molybdenum, and phosphorus metabolism reported that molybdenum administration causes a lowered liver copper value in cattle that are on an adequate but not excessive intake of copper. When cattle were on a low copper intake the depletion was marked. Conversely when cattle were on a high copper intake liver copper values were not lowered by molybdenum administration. Marston (72) has observed lowered liver copper values in sheep receiving molybdenum on a normal pasture. However, after having been treated with molybdenum for two years the copper level was still capable of fulfilling physiological requirements. When this experiment was repeated on a copper deficient pasture, the same amount of molybdenum decreased the rate of depletion of copper from the liver and maintained a higher concentration of copper in the blood, while at the same time producing

severe copper deficiency symptoms.

Conditioned Copper Deficiency in Ruminants: Molybdenum has been implicated as a conditioning factor in copper deficiency in ruminants on pastures containing normal or marginal levels of copper by a number of investigators (8, 14, 26, 28, 38, 72). The similarity in symptoms between conditioned copper deficiency attributed to molybdenum and that not attributed to excessive molybdenum has led to the opinion that a "factor" other than molybdenum may also exert an influence on the storage of copper in the liver (28, 30, 72). Bennetts et al. (10, 11) in reporting on the symptoms of "falling disease" of cattle in Western Australia have presented the clinical symptoms of this disease. Although the majority of the symptoms are those associated with copper deficiency there are some that would indicate that another factor is involved. The clinical symptoms were especially evident during the flush growth of pastures. Breeding difficulties were encountered in mature cattle and calves exhibited diarrhea and anemia. Symptoms reached maximum severity during late winter and spring. This period was followed by one in which there was an immediate and sharp rise in the hemoglobin values of both affected animals and those receiving copper supplements. This rise was not associated with an increase in copper content of the pastures.

Jamieson and Allcroft (55, 56, 57) have reported on a condition encountered around Caithness, Scotland in which an apparent copper deficiency occurs in animals grazing on pastures with adequate copper levels. The condition is referred to as "Caithness disease" and appears to affect only young stock. Anemia and hemosiderosis, which are commonly associated with copper deficiency, were absent. Affected

animals exhibited a stilted gait and poor condition which responded to copper therapy. Allcroft and Parker (1) and Allcroft (2) observed low blood copper values in dairy cows grazing pastures of normal copper content. Daily administration of copper sulfate improved the condition. Blakemore and Venn (13) have also observed low blood copper values in cattle grazing on pastures of normal copper content in East Anglia. In one herd, copper therapy did not bring about a satisfactory recovery, however, the further addition of cobalt brought about a complete remission. Similar conditions have been reported in certain sections of the north eastern part of the Netherlands. Here both simple and conditioned copper deficiencies have been observed (95). Pastures in this area were found to be low in cobalt and the affected animals showed a favorable response to the oral administration of cobalt or a mixture of copper and cobalt. The molybdenum content of grass from this area was around two p.p.m. in the dry matter and was not considered a conditioning factor. Conditioned copper deficiencies in sheep have been observed in Great Britain and referred to as "swayback". This and related conditions have been reviewed by Russell (89) and Marston (73).

Dick (30) has provided evidence for the existence of a factor in lucerne chaff which modifies the control of liver copper storage by molybdenum. Five groups of sheep were placed on oaten chaff, oaten and lucerne chaff mixtures, and lucerne chaff diets supplemented with copper and molybdenum to provide an equal intake for all groups. The oaten chaff diet resulted in an increase in the liver copper level and blood molybdenum level that was approximately ten times that observed in sheep on the lucerne chaff diet. Values for the other groups were

intermediate and proportional to the amount of oaten chaff present. The urinary excretion of molybdenum by sheep on the chaffed lucerne diet was much greater than for the other groups and probably accounted for the reduced level of molybdenum in the blood.

Molybdenum in the Nutrition of Monogastric Animals: Monogastric animals apparently have a much higher tolerance for molybdenum than do ruminants. Fairhall et al. (37) investigated the acute toxicity of molybdenum and found that doses of from 1200 to 6000 milligrams of molybdenum per kilogram of body weight in the form of molybdenum trioxide, calcium molybdate and ammonium molybdate were fatal when fed to rats and guinea pigs. Intraperitoneal injections of 400 to 800 milligrams of soluble molybdenum compounds per kilogram of body weight produced a high mortality in guinea pigs.

In 1948, Neilands et al. (79) reported on molybdenum in the nutrition of the rat. They observed that molybdenum at levels of 500 and 1000 p.p.m. inhibited growth to a marked degree. The inhibitory effects resulting from a level of 400 p.p.m. of molybdenum were largely overcome by supplemental copper. Whole liver substance was also capable of overcoming the inhibitory effects but neither iron, zinc, nor cobalt showed an appreciable effect. Comar et al. (21) reported on molybdenum metabolism and interrelationships with copper and phosphorus. In studies with weanling rats these investigators found that on a purified, low copper diet the addition of molybdenum at a level of eighty p.p.m. resulted in retarded skeletal growth with poor calcification, diarrhea, roughened hair coat and in severe cases an excessive lachrymation of the eyes. Molybdenum toxicity was observed to be less pronounced in the

mature rat. Gray and Ellis (45) also observed molybdenum toxicity in rats on both mineralized whole milk powder and commercial rat chow diets supplemented with molybdenum. Growth inhibition was the primary symptom of molybdenum toxicity. This effect was partially corrected by the addition of copper to the diet.

Jeter and Davis (58) in studying the effects of molybdenum on gestation and lactation in rats found that the addition of molybdenum at a level of eighty or 140 p.p.m. to a low copper diet produced a high incidence of sterility in males but not in females. Neither gestation nor parturition appeared to be affected by molybdenum, but there was a greater loss of weight by the female during lactation. At weaning, litters whose dams had been on a molybdenum supplemented diet prior to conception or received molybdenum after parturition weighed less than those from unsupplemented dams. From this evidence it would appear that molybdenum in the diet of the lactating female exerts a growth inhibiting effect on the nursing young.

Swine have also been used in molybdenum metabolism studies. This species apparently has a high tolerance for molybdenum (28). Kubisch (64) found that the growth inhibiting effect of molybdenum at a level of 1000 p.p.m. was much less in swine than in rats. Feed utilization was not significantly influenced by the addition of molybdenum to the diet. Histological examinations revealed no evidence of degenerative changes in the nervous system such as those encountered in sheep on copper deficient pastures (24, 25, 70, 72). Molybdenum accumulated in the liver, kidney, femur shaft, spleen and hide. The copper content of the liver, kidney, spleen, hide and blood plasma was greatly in-

creased. Molybdenum and copper accumulation in rats on high levels of molybdenum were essentially the same as observed in swine.

Arrington (4) used rabbits and rats in studying interrelationships of molybdenum, copper and phosphorus. Feeding high levels of molybdenum to rabbits resulted in the appearance of front leg abnormalities characterized by gross changes in the bone. Alopecia, diarrhea and anemia were also observed.

Kretzer (63) has extended molybdenum metabolism studies to chicks and poults. He reported that the growth of chicks and poults was depressed by approximately twenty five per cent by the addition of 300 p.p.m. molybdenum to their rations. The addition of copper to the rations containing molybdenum caused slightly improved growth. Anemia [illegible]

Use of Radioisotopes in Molybdenum Metabolism Studies: Radioisotopes of copper, molybdenum, calcium and phosphorus have been used in molybdenum metabolism studies. In 1948, Neilands, Strong and Elvehjem (79), reported on the use of molybdenum 99 in tissue distribution studies with rats. Orally administered radiomolybdenum was found to accumulate preferentially in the liver, kidney and skeleton. The simultaneous administration of copper did not influence the distribution of the molybdenum. However, the simultaneous administration of catechol resulted in a greater tissue accumulation of molybdenum. A relationship between catechol and molybdenum has also been proposed by McGowan and Brian (69).

Comar, Singer and Davis (21) reported on interaction studies with

the radioisotopes molybdenum 99, phosphorus 32 and copper 64. Radiomolybdenum distribution studies in cattle, following both oral and intravenous administration, showed a high selective accumulation in the adrenals, bone, liver, eye, intestinal lymph glands and kidneys. The distribution pattern of radiomolybdenum and radiophosphorus in cattle was very similar and suggested that molybdenum might exert a competitive effect toward phosphorus. Studies with rats indicated that when molybdenum and radiophosphorus are administered simultaneously that absorption of the phosphorus from the intestinal tract is increased, however, rats receiving a moderate level of molybdenum in the diet showed a decrease in the tissue accumulation of radiophosphorus. The simultaneous administration of molybdenum or phosphorus with radiocopper resulted in a reduced liver accumulation of radiocopper. This effect was also observed in calves that were on a molybdenum supplemented diet.

Shirley, Owens and Davis (96, 97) in studying the effects of copper and molybdenum on the excretion of radiophosphorus in rats and cattle observed that phosphorus excretion increased when molybdate was administered and there was also an increase in the fecal excretion of radiophosphorus. Arrington (4) has observed similar results in rabbits. These data tend to implicate molybdenum as a factor in phosphorus metabolism.

Kulwich (64) used radioisotopes of calcium, copper and molybdenum in studying some of the interrelationships of molybdenum, copper and zinc in the nutrition of rats and swine. Drenching weanling rats with

sodium molybdate for a short period prior to oral administration of radiocopper resulted in a greater retention of radiocopper in the blood and liver. Similar results were obtained with mature young rats following intramuscular injection of radiocopper. Radiomolybdenum was used in excretion studies with swine. Over fifty per cent of orally administered radiomolybdenum was excreted in the urine within twenty four hours. Radiocalcium studies with rats showed that molybdenum in the diet tended to increase the calcium turnover in the bones and to increase the urinary excretion of radiocalcium but at the same time decrease the fecal excretion of radiocalcium, the latter accounting for the largest proportion of excretion.

The use of radioisotopes in nutrition research has been reviewed by Comar (20, 22).

Possible Factors in Molybdenum Metabolism: Copper and molybdenum have been shown to be related in animal nutrition, however, it has not yet been established whether this is a direct or indirect relationship. The lack of a definite stoichiometrical relationship tends to favor the latter. If this is the case then it might be assumed that molybdenum exerts its effect on copper metabolism through interference with another factor which in turn is directly related to copper or to yet other factors which affect copper. This section of the literature review is devoted to a brief survey of factors which may be involved in molybdenum metabolism.

Molybdenum toxicity symptoms are similar in many respects to those resulting from a deficiency of certain water soluble vitamins.

Folic acid and pantothenic acid deficiency symptoms were found to resemble those of molybdenum toxicity in many respects. However, the conditions associated with the occurrence of molybdenum toxicity does not lead one to suspect a dietary deficiency of either of these vitamins. There remains the possibility that the conversion of these vitamins to their active forms or their subsequent utilization in intermediate metabolism may be effected by an excess of molybdenum in the diet.

Pantothenic acid deficiency in rats and mice has been associated with the development of alopecia (121), achromotrichia (98, 106), porphyrin caked whiskers (114, 115), impaired testicular function (5), hypoplasia of the epiphyseal cartilage (81), adrenal hemorrhage and necrosis (32, 90, 108), myelin degeneration of the sciatic nerve and spinal cord (67), inflammatory changes in the respiratory tract, lobular hepatitis, fatty infiltration of the liver (60), hypochromic anemia, lowered hemoglobin and hyperplasia of the bone marrow (16). How many of these symptoms can be attributed strictly to a deficiency of pantothenic acid is uncertain as many of the symptoms are associated with other members of the B vitamin complex. A close relationship between pantothenic acid and copper metabolism has been shown to exist. Singer and Davis (106) in studies with rats on a low copper diet observed that either pantothenic acid or copper would correct the achromotrichia which developed. They proposed that a copper deficiency might accentuate the pantothenic acid requirement of the rat. Handley and Ing (54) have reported that on a pantothenic acid deficient diet copper accumulated in the skin up to four times the level normally encountered in rats.

The accumulation of copper as a result of a vitamin deficiency is particularly interesting since both Marston (72) and Kulwich (64) have observed that an excess of molybdenum may cause an accumulation of copper in selected tissues of the rat. Yacowitz, Norris and Hauser (123) have established a relationship between pantothenic acid and Vitamin B_{12}. In studies with hens and chicks they observed that each of these vitamins exerted a sparing effect on the other and they proposed that Vitamin B_{12} may be involved in the conversion of pantothenic acid to coenzyme A. The importance of pantothenic acid in intermediary metabolism was demonstrated earlier by Hills (51) and Dorfman et al. (33). Biotin and folic acid have also been shown to be necessary for the utilization of pantothenic acid by the rat (34).

Although dietary deficiencies of the folic acid complex are unlikely under conditions associated with naturally occurring molybdenum toxicity, there remains the possibility that the utilization of the conjugated forms of folic acid or the conversion of folic acid to Leuconostoc citrovorum factor may be affected by excess molybdenum. The position of folic acid is somewhat unique in that there are several related compounds that possess varying degrees of folic acid activity for animals. The term folic acid is associated with the compound pteroylglutamic acid, which is active for animals. In addition there are the naturally occurring conjugates, pteroylheptaglutamic acid and pteroyltriglutamic acid. The former occurs in plants and the latter is a fermentation product. In order for animals to utilize the conjugate forms it is necessary that they be hydrolyzed to the monogluta-

mate form. This is brought about by the action of "folic acid conjugase" which Pfiffner and Hogan (84) have classified as a carboxypeptidase enzyme. The properties of "folic acid conjugase" have not yet been completely established, however, it is probably a metallo-enzyme. Bethell et al. (12) have reported that patients with pernicious anemia in relapse and post-gastrectomy macrocytic anemia cannot readily utilize for hematopoiesis a naturally occurring conjugate of pteroylglutamic acid although they respond promptly to administration of the free vitamin. Inability to utilize the conjugated vitamin appears to be dependent at least in part upon a conjugase inhibiting substance present in natural sources of conjugate. The administration of a liver fraction with the conjugate permitted its utilization. Soares et al. (105) have used both pteroyltriglutamic and pteroylheptaglutamic acid in the treatment of tropical sprue. The utilization of the conjugates was enhanced by the administration of a folic acid free liver extract. Swendseid et al (110) have observed that the urinary excretion of pteroylglutamic acid is reduced in normal patients when the conjugate form of the vitamin is administered with a conjugase inhibitor. Dietrich, Monson and Elvehjem (31) reported that the oral administration of vitamin B_{12} increased the conversion of pteroylglutamic acid to Leuconostoc citrovorum factor in the pteroylglutamic acid-vitamin B_{12} deficient chick. The results of these experiments suggest that vitamin B_{12} may be involved in the metabolism of folic acid conjugate and the conversion of pteroylglutamic acid to Leuconostoc citrovorum factor. Martin (74) and Wright and Welsh (122) have established folic acid as a

chromatrichial factor in rats. Spicer et al. (103) were able to correct the agranulocytosis, leucopenia, anemia and hypocellularity of the bone marrow of rats on a diet containing sulfa drugs by the addition of folic acid concentrate. Similar results were obtained by Endicott et al. (35). Cereceda and Vinson (17) have reported that concentrates of pteroylglutamic acid improved the performance of rats during lactation. This observation has been confirmed by Nelson and Evans (80). Nichol and Welch (82) have observed a rapid loss of body weight and a severe diarrhea in rats on a purified diet deficient in pteroylglutamic acid and supplemented with succinylsulfathiazole. Daniel, Farmer and Norris (27) reported that perosis in White Leghorn chicks varies with the amounts of folic acid in the diet. Schaefer et al. (92) have shown a relationship between choline, folic acid and vitamin B_{12} in the prevention of perosis in baby chicks. Folic acid and vitamin B_{12} were also essential for normal feathering and weight gains. Schweigert (94) has observed a weakness and inability to walk in young turkey poults on a folic acid deficient diet. Sunde et al. (107) in reproduction studies with chickens observed bone deformities in the newly hatched chick when the hen had been on a pteroylglutamic acid deficient ration. Pronounced weakness was also observed. Rodney et al. (88) have presented evidence to indicate that pteroylglutamic acid influences tyrosine oxidation "in vitro".

Ralli and Graaf (85, 86) have established a relationship between pantothenic acid and the adrenal cortex. Adrenalectomy provided a stimulus great enough to reverse the atrophy of the hair follicles and

depigmentation caused by feeding rats on a diet deficient in the filtrate factors of the vitamin B complex. Hundley and Ing (59) in copper deficiency studies with rats observed that adrenalectomy completely prevented development of gray hair in most of the animals. Either adrenalectomy or hypophysectomy in rats caused the hair coat which had become gray on a copper deficient diet to partially or completely return to a normal black. These findings indicate a metabolic relationship between copper and the adrenal cortex. The hormones of the adrenal cortex have been shown to be involved in the suppression of lymphoid tissue, hair and the epiphyseal cartilage of the tibia of rats (7). Evans, Simpson and Li (36) reported that injection of adrenocorticotropic hormone (ACTH) into male rats resulted in a retardation of chondrogenesis and osteogenesis in the region of the proximal epiphysis of the tibia. Growth inhibition was also observed. Baker and Ingle (6) have confirmed these results. Nelson et al. (81) have reported a similar impairment of chondrogenesis in rats on a pantothenic acid deficient diet. Winter, Silber and Steark (120) found that the administration of cortisone acetate to young adult rats caused a cessation of growth and suppressed regrowth of hair. Histological examination of sections of rib and tibia revealed a reduction in the width of the epiphyseal cartilage but no apparent rarification of cortical or cancellous bone. Follis (42, 43) has reported that cortisone acetate administered to rats causes skeletal changes but when administered to mice and guinea pigs in equivalent doses does not produce changes. Wahlstrom and Johnson (118) have reported that the administration of cortisone to young pigs on a

vitamin B_{12} deficient diet enhanced the deficiency. Meites (75) has reported that vitamin B_{12} counteracted the growth inhibiting effect of cortisone in immature male rats.

[redacted]

Certain analytical procedures have been used in a number of the experiments reported on herein and in order to avoid repetition the details and references for these procedures are presented in this section. In addition, radioisotope procedures used in Experiment 6 are also pre-[redacted]

Copper analysis was carried out on feed and liver samples. Approximately one gram samples of air dry feed and oven dried (sixty-five degrees centigrade for twenty four hours) liver were used. The samples were placed in 100-milliliter beakers, covered with suitable watch glasses and wet-ashed according to the method of Lindner and Harvey (65). Several additions of thirty per cent hydrogen peroxide, followed by further digestion, were found necessary to achieve complete oxidation of all organic material present in the samples. After cooling, the samples were transferred to twenty-five milliliter volumetric flasks and made to volume with pyrex distilled water. Blanks and standards used in the determination were accorded the same treatment as the samples. The carbamate method (91), modified by the use of isoamyl alcohol (19) as the solvent for extracting the copper diethyldithiocarbamate complex, was used. Transmittancy was determined in a Beckman Model B Spectrophotometer at 444 millimicrons with the blank set at 100 per cent trans-[redacted]

Table 1. presents a summary of the microbiological methods used

Table 1

Methods for the Microbiological Determination of Five Members of the Vitamin-B Complex

Vitamin Determined	Test Organism[1]	Reference
Nicotinic acid	Lactobacillus arabinosus 17-5	Snell and Wright (102)
Pantothenic acid	Lactobacillus arabinosus 17-5	Skeggs and Wright (99)
Riboflavin	Lactobacillus casei c	Snell and Strong (101)
Folic acid	Streptococcus lactis R	Cappa, Hobbe, and [illegible] (15)
Vitamin B_{12}	Lactobacillus leichmannii 4797	Thompson, Dietrich and Elvehjem (113)

[1] All microorganisms used in these determinations were obtained from the American Type Culture Collection, 2029 M Street, N.W. Washington 6, D. C.

Table 2

Properties of Radioisotopes of Calcium and Molybdenum

Isotope	Half-life	Radiation Energy (mev) Beta	Gamma	Projectile and Target		Specific Activity Initially Available Millicuries/gram
[illegible]	152 days	0.26	[illegible]	Neutrons $CaCl_2$ $CaCO_3$	solution	5-20
Mo^{99}	67 hours	1.30	0.77, 0.815, 0.81?	Neutrons MoO_3	MoO_3	7

for B-vitamin determinations.

Radioactive materials were handled according to the instructions set forth in National Bureau of Standards Handbook 42 (3) in order to minimize health hazards. Radioisotopes of calcium and molybdenum were used to trace the metabolism of these elements in young beef calves under the conditions of Experiment 6. The properties of these isotopes are presented in Table 2. (119)

The calcium 45 was received as a solution of calcium chloride which was diluted to an appropriate volume with distilled water and adjusted to a pH of 6.5 with 0.1 normal sodium hydroxide. The final solution contained 40.98 milligrams of calcium per milliliter with an estimated activity of 82.13 microcuries per milliliter. Each calf used in the radiocalcium study was given fifteen milliliters of this solution, containing an estimated activity of 1.23 millicuries, by intravenous injection. The calcium 45 reference standard was prepared from this solution by diluting 0.1 milliliter to 100 milliliters with distilled water and then transferring 0.5 milliliter of the diluted solution to a stainless steel cup 2.5 centimeters in diameter and 0.5 centimeter in depth. The solution in the cup was evaporated to dryness under an infra red lamp and stored in a desiccator. Blood plasma samples were prepared for counting by transferring 0.5 milliliter of plasma to a stainless steel cup of the type described and evaporated to dryness under an infra red lamp. Corrections for self absorption were made. Tissue, feces and urine samples were prepared for counting by the method of Comar, et al. (23). Calcium 45 samples were counted in a fully

shielded chamber using a thin mica-window counter tube.

The molybdenum 99 was received as an irradiation unit containing ten grams of molybdenum trioxide. The material as received was dissolved in sodium hydroxide with the aid of a mechanical stirrer according to the method of Comar, et al. (21). The final solution was adjusted to pH 7.0 with hydrochloric acid and made to a final volume of fifty milliliters with distilled water. Each calf used in the radiomolybdenum study was given thirteen milliliters of the solution, containing an estimated initial activity of 4.43 millicuries, by intravenous injection.

Separate molybdenum 99 reference standards were prepared for tissue, blood and plasma concentration determinations. The tissue reference standard, which was also used in determining the concentration in the feces and urine, was prepared by diluting 0.25 milliliter of the prepared solution so that, when one milliliter of the final solution was transferred to a sixty centimeter diameter Petri dish and diluted by the addition of nine milliliters of distilled water, the final solution in the dish had an activity that was within the normal counting range of the instrument used. Reference standards for blood and plasma determinations were prepared by adding one milliliter of a suitably diluted solution containing the molybdenum 99 to nine milliliters of neutral blood and plasma respectively. After thorough mixing one milliliter of each was transferred to stainless steel cups measuring 2.5 centimeters in diameter and 0.5 centimeter in depth. Liquid reference standards were counted immediately after preparation in order to avoid any changes in the dispersion of the molybdenum 99. Tissue, feces and urine samples

were wet-ashed in nitric acid. Ten milliliter aliquots were transferred to a sixty centimeter Petri dish for counting. One milliliter samples of blood and plasma were transferred to stainless steel cups and counted directly. Molybdenum 99 samples were counted in a partially shielded chamber using a thin mica-window counter tube. Decay rate corrections were applied to all molybdenum 99 determinations.

Tissues to be used for histological studies were removed from the animal immediately after sacrifice and placed in a ten per cent formalin solution. After a suitable fixation period, osseous tissues were removed, dehydrated in seventy per cent ethyl alcohol and decalcified in ten per cent nitric acid. Once decalcified, the osseous tissues were handled in the same manner as the soft tissues. The procedures employed in preparation, embedding, sectioning and mounting were essentially those described by Jones and McClung (59). The mounted sections were stained with hematoxylin and counterstained with eosin.

EXPERIMENT 1

Effect of Three Different Molybdenum Compounds on Growth and B Vitamin Utilization by Young Rats

Molybdenum toxicity in grazing ruminants appears to be associated with factors in addition to the amount of molybdenum in the forage. Mature forage has caused less trouble than actively growing forage even though the former contains a higher total concentration of molybdenum (8). It has been suggested that the form in which molybdenum is present in forage may be related to its toxicity (26). This experiment was undertaken in order to determine the effects of three different molybdenum compounds on growth of rats and utilization of selected members of the Vitamin B complex. A secondary objective was the development of procedures and techniques that could be used in subsequent experiments.

Thirty young rats from a highly inbred Wistar strain averaging forty days old, were randomized and divided into five groups, with three males and three females in each group. Group I (low copper) was placed on a Ca-3 basal ration (see Table 3 for composition) modified by omission of copper sulfate and yeast. Group 2 (control) was fed the modified basal ration supplemented with 7.86 milligrams of copper sulfate per kilogram. Groups 3, 4 and 5 were fed the modified basal ration supplemented with 7.86 milligrams of copper sulfate and 1.29 grams of sodium molybdate, 0.92 grams of ammonium molybdate and 0.75 grams of molybdenum trioxide per kilogram respectively. Pyrex distilled water was provided for all groups. The analytical value of copper obtained for the modified basal ration was 3.1 parts per million (p.p.m.). Kulvich (64) reported the analytical value for molybdenum in the Ca-3 basal ration as less than 1.0 p.p.m. All animals were weighed weekly.

After five weeks on experimental rations all rats were transferred to individual metabolism cages which permitted separation and quantitative collection of feces and urine. A twenty-four hour fasting urinary excretion collection was made. Urine was transferred from collection tubes to sample bottles containing one milliliter of toluene at four hour intervals in order to minimize bacterial growth. Urine samples were held in a refrigerator at a temperature of approximately eight degrees centigrade until analyzed. All urine samples were made to a volume of 100 milliliters with distilled water and aliquots were

Table 3

University of Tennessee - Atomic Energy Commission Agricultural Research Program Ca-3 Purified Rat Ration

Ingredients	
Corn Starch	642.6 grams
Casein	180.0 grams
Irradiated Yeast	10.0 grams
$NH_4H_2PO_4$	13.2 grams
$CaCO_3$	12.2 grams
UT-AEC Mineral Mix I[1]	20.0 grams
Wesson Oil	40.0 grams
Vitab[2]	40.0 grams
Cod Liver Oil	2.0 grams
Cellulose	40.0 grams
	1,000.0 grams

[1] UT-AEC Mineral Mix I

NaCl	390.4 grams
KI	182.0 grams
$KHCO_3$	262.0 grams
$MgSO_4$	120.0 grams
$CaSO_4 \cdot 2H_2O$	4.0 grams
$MnSO_4 \cdot 4H_2O$	2.0 grams
$ZnSO_4 \cdot 7H_2O$	6.0 grams
$FeCl_3 \cdot 6H_2O$	25.0 grams
$K_2Al_2(SO_4)_4 \cdot 24H_2O$	0.2 grams
KI	0.3 grams
NaF	0.1 grams
	1,000.0 grams

[2] Vitab fortified with 400 milligrams of riboflavin per kilogram (from Hopco Chemical Company, Harrison, New Jersey).

taken for vitamin determinations. Pantothenic acid and folic acid were determined microbiologically.

During the sixth week of the experiment all male rats were given intramuscular injections of five micrograms of Teropterin[1], placed in individual metabolism cages and urine collections made for the next two succeeding twelve hour periods. Microbiological assays for folic acid were run on diluted aliquots of the urine samples. During the seventh week of the experiment all rats were administered twenty five micrograms of Teropterin by intramuscular injection. Two hours after administration of Teropterin a blood sample of approximately 0.015 cubic centimeter was taken in a Thoma leucocyte diluting pipette from the clipped tail of each rat. The samples were diluted with twenty volumes of distilled water, placed in centrifuge tubes and stored in a refrigerator at a temperature of approximately eight degrees centigrade until analyzed. For analysis the diluted blood samples were further diluted by the addition of 9.7 milliliters of distilled water. In order to remove blood proteins the diluted samples were autoclaved at fifteen pounds pressure for five minutes and after cooling, centrifuged. Folic acid was determined microbiologically. The experiment was terminated at the end of the eighth week.

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[1] Teropterin (pteroyltriglutamic acid) was furnished through the kindness of Lederle Laboratories, Pearl River, New York.

RESULTS

The typical symptoms of molybdenum toxicity observed in rats, i.e., depressed growth, achromotrichia and alopecia were not apparent in any of the experimental groups. The weight gains are presented in Table 4. Under the conditions of this experiment the three forms of molybdenum fed apparently had no appreciable effect on weight gain.

The fasting urinary excretion values of folic acid and pantothenic acid for a twenty-four hour period during the fifth week of the experiment are presented in Table 5. In the male rats there appeared to be a tendency for a higher excretion of folic acid in the low copper and molybdate supplemented groups than in the control group and molybdenum trioxide supplemented group. A similar trend was observed in the females with the exception that the low copper group excreted less folic acid than did the control group. There appeared to be a considerable individual variation as well as a sex difference in the excretion of pantothenic acid. The higher excretion of pantothenic acid by females in the low copper and control groups is the most consistent finding.

With one exception, all of the male rats receiving an intramuscular injection of five micrograms of Teropterin showed an increase in urinary excretion of folic acid over that observed during the previous week. Excretion levels were higher during the first twelve hours following injection than during the second twelve hour period. The increase in excretion of folic acid was fairly consistent for all groups with the exception of two individuals. The intramuscular injection of twenty

Table 4

Effect of Varying Levels of Copper and Molybdenum in Purified Rations on the Growth of Young Rats

Group Number	Treatment	Sex	Rat Number	Initial weight (grams)	Gain 4 weeks (grams)	Gain 8 weeks (grams)
1	Basal Ration	Male	1	107	87	141
		Male	2	100	88	135
		Male	3	144	120	183
	Average			117	98	153
		Female	4	103	49	*
		Female	5	101	34	83
		Female	6	55	75	130
	Average			86	53	76
2	Basal Ration + 2 p.p.m. Copper	Male	2	93	89	117
		Male	3	127	83	143
		Male	4	123	84	155
	Average			114	85	138
		Female	5	113	*	
		Female	6	87	57	86
		Female	7	87	90	134
	Average			82	74	110

Table 4 (Continued)

Effect of Varying Levels of Copper and Molybdenum in Purified Rations on the Growth of Young Rats

Group Number	Treatment	Sex	Rat Number	Initial weight (grams)	Gain 4 weeks (grams)	Gain 8 weeks (grams)
3	Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as sodium molybdate	Male	1	81	89	*
		Male	3	85	*	
		Male	4	117	91	132
	Average			94	90	139
		Female	5	61	58	87
		Female	6	90	60	84
		Female	7	116	58	77
	Average			89	59	83
4	Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as ammonium molybdate	Male	2	97	119	184
		Male	3	115	67	81
		Male	4	76	67	*
	Average			96	84	133
		Female	5	80	73	85
		Female	6	74	72	116
		Female	7	82	60	91
	Average			79	68	97

Table 4 (Continued)

Effect of [illegible] Levels of Copper and Molybdenum in Purified Rations [illegible] of Young Rats

Group Number	Treatment	Sex	Rat Number	Initial weight (grams)	Gain 4 weeks (grams)	Gain 8 weeks (grams)
5	Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as molybdenum trioxide	Male	1	107	84	135
		Male	2	117	80	130
		Male	3	89	84	150
	Average			104	83	138
		Female	5	79	90	123
		Female	6	75	52	72
		Female	7	114	18	64
	Average			89	53	86

* Deaths.

Table 5

Effect of Varying Levels of Copper and Molybdenum on the Fasting Urinary Excretion of Folic Acid (FA) and Pantothenic Acid (PA) by Young Rats in a Twenty Four Hour Period

Treatment	Micrograms FA and PA per 100 grams body weight					
	Male			Female		
	Rat Number	FA	PA	Rat Number	FA	PA
			Group 1			
Basal Ration	1	0.106	4.369	4	0.053	12.132
	2	0.071	6.430	5	0.053	8.023
	3	0.040	3.857	6	0.025	17.361
Average		0.072	4.888		0.044	12.477
			Group 2			
Basal Ration + 2 p.p.m. Copper	2	0.030	9.062	6	0.065	11.623
	3	0.034	5.089	7	0.059	11.093
	4	0.029	4.655			
Average		0.031	6.269		0.062	11.358
			Group 3			
Basal Ration + 2 p.p.m. Copper + 500 p.p.m. Molybdenum as sodium molybdate	1	0.051	3.378	5	0.077	
	4	0.060	11.079	6	0.146	5.365
					0.065	5.539
Average		0.056	7.209		0.096	5.452
			Group 4			
Basal Ration + 2 p.p.m. Copper + 500 p.p.m. Molybdenum as ammonium molybdate	2	0.341	12.913	5	0.204	10.192
	3	0.052	12.379	6	0.078	6.187
					0.372	3.785
Average		0.197	12.640		0.218	6.701

Table 5 (Continued)

Effect of Varying Levels of Copper and Molybdenum on the Fasting Urinary Excretion of Folic Acid (FA) and Pantothenic Acid (PA) by Young Rats in a Twenty Four Hour Period

	Micrograms FA and PA per 100 grams body weight					
		Male			Female	
Treatment	Rat Number	FA	PA	Rat Number	FA	PA
Basal Ration + 2 p.p.m. Copper + 500 p.p.m. Molybdenum as molybdenum trioxide	1	0.071	17.960	5	0.323	7.277
	3	0.021	3.697	6	0.021	2.997
	4	0.035	2.995	7	0.079	3.589
Average		0.042	8.214		0.141	4.921

five micrograms of Teropterin caused a rapid increase in the level of free folic acid in the blood within two hours. This increase was fairly consistent throughout all groups. The average folic acid level of the blood prior to injection of Teropterin was 2.8 millimicrograms per milliliter. The detailed results of these tests are presented in Tables 6 and 7.

Table 6

Effect of Varying Levels of Copper and Molybdenum on the Fasting Urinary Excretion of Folic Acid (FA) by Young Male Rats Following Intramuscular Injection of Five Micrograms of "Teropterin"[1]

Treatment	Rat Number	Milligrams FA per 100 grams body weight: First 12 Hours	Second 12 Hours	24 Hours
	Group 1			
Basal Ration	1	79	28	107
	2	45	24	69
	3	63	33	96
Average		61.6	28.3	90
	Group 2			
Basal Ration + 2 p.p.m. Copper	2	22	12	34
	3	39	25	64
	4	43	42	85
Average		34.7	26.3	61
	Group 3			
Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as sodium molybdate	1	61	38	99
	4	33	37	70
[illegible]		47	37.5	84.5
	Group 4			
Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as ammonium molybdate	2	192	143	335
	3	62	24	86
Average		127	83.5	210.5

Effect of Varying Levels of Copper and Molybdenum on the Fasting Urinary Excretion of Folic Acid (FA) by Young Male Rats Following Intramuscular Injection of Five Micrograms of "Teropterin"[1]

Treatment	Rat Number	Millimicrograms FA per 100 grams body weight		
		First 12 Hours	Second 12 Hours	24 Hours
	Group 5			
Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as molybdenum trioxide	1	66	57	123
	3	49	44	93
	4	42	37	86
Average		54.6	46	101

[1] Teropterin = pteroyltriglutamic acid

Table 7

Effect of Varying Levels of Copper and Molybdenum on the Blood Folic Acid (FA) Level in Young Rats Two Hours After Intramuscular Injection of Twenty Five Micrograms of Pteropterin[1]

Group Number	Treatment	Rat Number	Micrograms of FA per milliliter of Blood
1	Basal Ration	1	1.0
		2	1.3
		3	0.1
		4	*
		5	1.5
		6	1.5
2	Basal Ration + 2 p.p.m. Copper	2	1.3
		3	1.2
		4	1.6
		5	*
		6	1.6
		7	1.3
3	Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as sodium molybdate	1	1.2
		4	1.3
		5	1.5
		6	1.3
		7	1.0
4	Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as ammonium molybdate	2	2.8
		3	1.1
		4	*
		5	1.1
		6	1.0
		7	0.7
5	Basal Ration + 2 p.p.m. Copper and 500 p.p.m. Molybdenum as molybdenum trioxide	1	1.0
		3	1.0
		4	0.8
		5	1.0
		6	0.2
		7	0.2

*Values less than 0.1 microgram of FA per milliliter of blood.

[1] Pteropterin = pteroyltriglutamic acid

DISCUSSION

The results of this experiment were somewhat unanticipated. The failure to produce the clinical symptoms (21, 45, 64, 79) of molybdenum toxicity at a level of 500 p.p.m. of molybdenum might be attributed to age and previous diet. All rats had been weaned at twenty eight days of age and maintained on a stock diet until placed on experimental rations twelve days later.

A number of deaths occurred from unknown causes in both control and treated groups. All of the animals that died exhibited similar symptoms which included: sores around the mouth, blood colored exudates around the nose, lung congestion and rapid loss of weight for about two weeks followed by death. Similar symptoms have been observed in pantothenic acid deficient rats by Sure and Ford (109). The pantothenic acid level of the ration was determined as 14.2 micrograms per gram which is in excess of the requirement of the growing rat (109). The folic acid level of the ration determined as free folic acid was 0.5 microgram per gram.

The urinary excretion of pantothenic acid varied considerably between individuals, sex and treatments. The females showed higher levels of excretion on the molybdenum free diets whereas this was not the case for the males. The ranges observed within groups causes one to question the value of this technique for measuring the pantothenic acid status of the individual. The urinary excretion of folic acid varied considerably but to a lesser degree than did the pantothenic

acid. Although the rats were maintained in wire bottom cages coprophagy may well have been a contributing factor to the individual variations observed. The lack of any apparent inhibition of the conversion of Teropterin (pteroyltriglutamic acid) to free folic acid (pteroylglutamic acid) as a result of molybdenum supplementation suggests that molybdenum does not interfere with the "folic acid conjugase" enzyme system. However, there remains the possibility that molybdenum may interfere with the subsequent utilization of folic acid.

CONCLUSIONS

The failure to produce the clinical symptoms of molybdenum toxicity by feeding molybdenum at a level of 500 p.p.m. in the form of sodium molybdate, ammonium molybdate and molybdenum trioxide precludes the possibility of any definite conclusion concerning the relative toxicity of these three compounds under the conditions of this experiment.

The pantothenic acid excretion study indicated that the metabolism of this vitamin may be affected by an excess of molybdenum in the diet. Molybdenum at the level fed apparently had no appreciable effect on the enzymatic conversion of pteropterin to free folic acid.

The Effect of Molybdenum on Liver Copper and Vitamin B_{12} Concentration and on Endochondral Ossification in the Tibia of Young Rats.

An excess of molybdenum in the diet has been shown to result in an altered copper metabolism that is reflected by a change in the liver copper concentration. Marston (72) and Kubisch (64) have observed that a high level of molybdenum, in a diet containing adequate copper, caused an increased concentration of copper in the tissues of rats. Similar results were obtained in studies with swine (64). Handley and Ing (54) have reported that one of the consequences of pantothenic acid deficiency in rats was an increased concentration of copper in the tissues. Nelson et al. (81) have observed that pantothenic acid deficiency in young rats was also reflected by changes in the endochondral ossification of the tibia. Davis (28) and Cunningham (26) have reported that an excess of molybdenum superimposed on a diet deficient in copper resulted in an increased incidence of fractures in the bones of young cattle. The results obtained in Experiment 1. suggested that molybdenum may interfere with the metabolism of pantothenic acid. Iacovits et al. (123) have postulated a relationship between vitamin B_{12} and the conversion of pantothenic acid to coenzyme A.

This experiment was designed to determine the effect of molybdenum on growth, liver copper and vitamin B_{12} concentrations; liver weight to body weight ratio as an indication of vitamin B_{12} status and bone formation in the young rat.

[redacted]

Twenty six Piebald rats, averaging thirty days old, were randomised and divided into two groups. Each group contained ten males and three females. Group 1 (control) was placed on a Cu-3 basal ration (see Table 3 for composition) modified by reducing the copper from 4.0 grams to 0.393 grams of copper sulfate per kilogram of mineral mix. The addition of this amount of copper provided a total level of 5 p.p.m. in the ration. Group 2 was fed the modified basal ration supplemented with 2.064 grams of sodium molybdate per kilogram. Pyrex distilled water was provided ad libitum in both groups. All animals were weighed weekly. The experiment was terminated after forty days at which time all animals were sacrificed by exsanguination under ether anaesthesia. The livers were removed and weighed immediately after sacrifice and stored in a deep freeze until analysed for copper and vitamin B_{12} activity. Tibias were placed in a ten per cent formalin solution for fixation.

RESULTS AND DISCUSSION

The typical symptoms of molybdenum toxicity were produced within three weeks. Symmetrical alopecia and depressed growth were observed in both male and female rats. The alopecia developed over the shoulders as shown in Figure 1, and spread back along both sides in a symmetrical pattern. There was no evidence of alopecia in the control group. The effect of molybdenum on the growth of young rats is presented in Table 8. The molybdenum supplemented rats showed an apparent reduction in weight gains. The statistical significance of this effect was not determined because of the unexpected wide range in weight gains observed in the molybdenum supplemented male rats.

Table 9 presents the effect of molybdenum on the concentration of copper and vitamin B_{12} in the livers of young rats. The liver copper concentration observed in the molybdenum supplemented male rats was greater than that observed in the rats on the basal ration. The increased concentration was significant at the 95 per cent confidence level as determined by "Student's t test" (100). This observation is in agreement with those reported by Marston (71) and Kubisch (64). There was no apparent correlation between the liver copper concentration and the vitamin B_{12} concentration. Molybdenum supplementation did not cause a significant difference in vitamin B_{12} concentration. The vitamin B_{12} levels observed were essentially in agreement with those reported by Schlad *et al.* (93).

The liver weight to body weight ratios observed in all of the rats are presented in Table 10. There was no significant difference in

Figure 1. Control (left) and molybdenum supplemented (right) rats after twenty five days on experimental rations. Note the alopecia on the neck and shoulders of the molybdenum supplemented rat.

Table 8

The Effect of Molybdenum on the Growth of Young Piebald Rats on a Purified Ration for Forty Days

Rat Number	Sex	Initial Weight (grams)	Final Weight (grams)	Weight Gain (grams)
		Basal Ration		
1	Male	30	146	116
2	Male	42	196	154
3	Male	59	200	141
4	Male	36	142	106
5	Male	52	214	162
6	Male	55	220	165
7	Male	31	114	83
8	Male	48	210	162
9	Male	44	193	149
10	Male	42	144	135
	Average	43	178	135 ± 28.4 [1]
1	Female	29	127	98
2	Female	25	137	112
3	Female	32	156	117
	Average	31	140	109 ± 9.8
		Basal Ration + 500 p.p.m. Mo as sodium molybdate		
1	Male	35	36	1
2	Male	37	55	18
3	Male	47	159	112
4	Male	69	188	119
5	Male	32	*	
6	Male	48	139	91
7	Male	37	86	49
8	Male	31	85	54
9	Male	30	64	34
10	Male	45	150	105
	Average	41	107	65 ± 43.4

Table 8 (Continued)

The Effect of Molybdenum on the Growth of Young Piebald Rats on a Purified Ration for Forty Days

Rat Number	Sex	Initial Weight (grams)	Final Weight (grams)	Weight Gain (grams)
1	Female	30	65	35
2	Female	47	132	85
3	Female	34	80	46
Average		37	92	55 ± 27.1[1]

[1] Standard deviation of the mean.

* Animal died during second week of experiment.

Table 9

Effect of Molybdenum on Liver Copper and Vitamin B_{12} Activity Concentration in Young Rats on a Purified Ration for Forty Days

Rat Number	Sex	Micrograms of copper per gram liver, dry weight	Millimicrograms of vitamin B_{12} activity per gram liver, fresh weight
		Basal Ration	
1	Male	27.38	48.2
2	Male	21.04	42.1
3	Male	25.34	28.4
4	Male	20.87	39.4
5	Male	16.13	36.7
6	Male	22.40	60.1
7	Male	25.64	47.8
8	Male	27.76	37.4
9	Male	23.49	45.0
10	Male	20.39	28.4
Average		23.03 ± 3.61 [1]	41.4 ± 9.6
1	Female	20.26	32.0
2	Female	24.59	27.8
3	Female	25.58	27.0
Average		23.48 ± 2.82	22.2 ± 8.9
		Basal Ration + 800 p.p.m. Molybdenum as [illegible] molybdate	
1	Male	36.83	35.6
2	[illegible]	39.32	52.4
3	[illegible]	51.80	48.3
4	[illegible]	41.70	28.8
6	[illegible]	54.87	45.6
7	Male	35.33	42.5
8	[illegible]	44.40	60.0
9	[illegible]	37.44	43.5
10	[illegible]	43.10	37.6
Average		42.75 ± 7.35	43.8 ± 9.3

Table 9 (Continued)

Effect of Molybdenum on Liver Copper and Vitamin B_{12} Activity Concentration in Young Rats on a Purified Ration for Forty Days

Rat Number	Sex	Micrograms of copper per gram liver, dry weight	Millimicrograms of vitamin B_{12} activity per gram liver, fresh weight
1	Female	34.65	48.5
2	Female	31.85	59.0
3	Female	39.18	41.7
Average		35.22 ± 9.95	49.7 ± 8.7

[1] Standard deviation of the mean.

Table 10

Effect of Molybdenum on the Body to Liver Weight Ratio in Young Rats on a Purified Ration for Forty Days

Rat Number	Sex	Final Body Weight (grams)	Liver Weight (grams)	Liver weight per cent of body weight
		Basal Ration		
1	Male	146	6.643	4.55
2	Male	196	8.266	4.22
3	Male	200	8.928	4.46
4	Male	142	6.538	4.61
5	Male	214	7.808	3.65
6	Male	220	8.756	3.98
7	Male	114	5.508	4.83
8	Male	210	9.818	4.68
9	Male	193	11.383	5.79
10	Male	144	7.451	5.18
	Average	176	8.090	4.59 ± 0.60 [1]
1	Female	127	7.866	6.19
2	Female	137	7.193	5.25
3	Female	156	6.633	4.24
	Average	140	7.231	5.23 ± 0.98
		Basal Ration + 800 p.p.m. Molybdenum as sodium molybdate		
1	Male	36	1.517	4.21
2	Male	55	2.229	4.05
3	Male	159	6.856	4.31
4	Male	188	9.163	4.88
6	Male	139	6.779	4.88
7	Male	86	4.566	5.31
8	Male	85	3.626	4.27
9	Male	64	3.493	5.46
10	Male	150	7.468	4.98
	Average	107	5.077	4.71 ± 0.51

Table 10 (Continued)

Effect of Molybdenum on the Body to Liver Weight Ratio in Young Rats on a Purified Ration for Forty Days

Rat Number	Sex	Final Body Weight (grams)	Liver Weight (grams)	Liver weight per cent of body weight
1	Female	65	2.977	4.58
2	Female	132	6.824	5.17
3	Female	80	4.379	5.47
	Average	92	4.726	5.07 ± 0.43[1]

[1] Standard deviation of the mean.

the ratios as a result of molybdenum supplementation. Ling and Chow (66) and Schied et al. (93) in studies with vitamin B_{12} deficient rats observed that the liver weight to body weight ratios were relatively greater for the deficient rats. This observation suggested that vitamin B_{12} deficiency, as reflected by a change in the liver weight to body weight ratio, was not brought about as a result of molybdenum supplementation under the conditions of this experiment.

Histological examinations of the tibia from five of the males and three females in each group revealed no significant changes in endochondral ossification such as those described by Nelson et al. (81). There appeared to be a slight increase in width of trabeculae in the primary spongiosa, however, this was considered within the normal range.

CONCLUSIONS

The results obtained in this experiment indicated that:

(1) Growth retardation and alopecia are symptoms of molybdenum toxicity in young rats.

(2) An excess of molybdenum in a diet containing adequate copper may cause an increased concentration of copper in the livers of rats.

(3) There was no apparent correlation between liver copper and vitamin B_{12} concentrations.

(4) Dietary molybdenum did not appear to affect the concentration of vitamin B_{12} activity in the liver of the rat.

(5) Molybdenum did not appear to affect the function of pantothenic acid in bone formation.

EXPERIMENT 3

The Effect of Intramuscularly Administered Vitamin B_{12} on Molybdenum Toxicity in Young Female Rats

Naturally occurring factors other than copper, capable of alleviating molybdenum toxicity, have been shown to be present in liver (79) and lucerne hay (30). The high vitamin B_{12} potency of beef liver suggested that this vitamin may be the active principle responsible for the alleviation of molybdenum toxicity symptoms in the rat.

This experiment was designed to determine the effect of intramuscularly administered vitamin B_{12} on molybdenum toxicity in young female rats.

Thirty female albino rats, from a Carworth strain, averaging thirty days old were randomised and divided into three groups. Group 1 (control) was fed the modified Ca-3 basal ration described in Experiment 2. Group 2 was fed the modified Ca-3 basal ration supplemented with 2.064 grams of sodium molybdate per kilogram. Group 3 was fed the same molybdenum supplemented ration as Group 2 but was also given 0.4 micrograms of vitamin B_{12} per animal bi-weekly by intramuscular injection. Pyrex distilled water was provided ad libitum for all groups. All animals were weighed weekly. The experiment was terminated at the end of the eighth week at which time all animals were sacrificed by exsanguination under ether anaesthesia. The livers were removed, weighed immediately and stored in a deep freeze until analysed for copper.

RESULTS AND DISCUSSION

Molybdenum at a level of 800 p.p.m. in the ration caused a marked alopecia in the young female rats of Group 2 within two to three weeks. The development of the alopecial pattern was similar to that observed in Experiment 1. Bi-weekly intramuscular injections of 0.4 microgram of vitamin B_{12} per animal completely prevented the appearance of alopecia in the molybdenum supplemented rats of Group 3. Alopecia was not observed in the control group. The results obtained suggested that vitamin B_{12} may be an anti-alopecial factor for molybdenum induced alopecia.

The effect of molybdenum on growth and the effect of vitamin B_{12} injections on growth retardation resulting from molybdenum are presented in Table 11. Molybdenum produced a marked retardation of growth during the first four weeks of the experiment that was statistically significant at the ninety-nine per cent confidence level as determined by 'Student's t test' (100). However, there was no significant effect on growth, as measured by weight gain, between the fourth and eighth week of the experiment. The injection of vitamin B_{12} appeared to provide a partial alleviation of the growth depressing effect of molybdenum during the first four weeks. This effect of vitamin B_{12} was significant between the eighty and ninety per cent confidence level. The failure of molybdenum to continue to exert a marked growth depressing effect after the fourth week suggested that the rat is capable of adapting itself to a high dietary level of molybdenum. The mechanism of this physiological adaptation is not understood.

Table II

The Effect of Intramuscularly Administered Vitamin B_{12} on Molybdenum Induced Growth Retardation in Young Female Albino Rats.

Rat Number	Initial Weight (grams)	Gain 0-4 weeks (grams)	Gain 4-8 weeks (grams)
Basal Ration			
1	53	93	88
2	60	80	63
3	68	78	58
4	51	81	50
5	53	99	54
6	54	90	45
7	53	107	59
8	56	98	88
9	54	96	75
10	52	97	90
Average	53	92 ± 9.5[1]	64 ± 15.1
Basal Ration + 800 p.p.m. of Molybdenum as sodium molybdate			
1	52	68	40
2	58	76	34
3	58	58	32
4	57	79	28
5	56	94	37
6	44	86	38
7	45	83	42
8	44	18	23
9	39	39	28
10	38	60	38
Average	51	60 ± 25.2	36 ± 9.9

Table 11 (Continued)

The Effect of Intramuscularly Administered Vitamin B_{12} on Molybdenum Induced Growth Retardation in Young Female Albino Rats.

Rat Number	Initial Weight (grams)	Gain 0-4 weeks (grams)	Gain 4-8 weeks (grams)
	Basal Ration + 600 p.p.m. of Molybdenum as sodium molybdate + 0.4 micrograms of Vitamin B_{12} per animal twice weekly		
1	42	78	52
2	38	80	48
3	37	84	59
4	38	90	60
5	32	73	45
6	33	66	35
7	40	78	32
8	46	82	54
9	36	64	66
10	42	84	36
	Average 53	74 ± 12.8	46 ± 12.9

[1]Standard deviation of the mean.

The average fresh weights of the livers for the three groups after sacrifice were 5.67, 4.78 and 5.77 grams respectively. The average liver weight to body weight ratios expressed in per cent, for the three groups were as follows: Group 1, 3.20; Group 2, 3.23; Group 3, 3.33. The results of this experiment indicated that neither molybdenum, nor molybdenum and vitamin B_{12} had an effect on the liver weight body weight ratio. Similar results were observed in Experiment 2.

Table 12 presents the effects of molybdenum supplementation with and without vitamin B_{12} injections on the concentration of copper in the liver. There was no significant difference in the concentration of copper in the liver as a result of the treatments. This is not in agreement with the results observed in male rats in Experiment 2. Thus suggesting that sex difference may have an effect on liver copper concentration in molybdenum supplemented rats.

Table 12

The Effect of Molybdenum with and without Vitamin B_{12} Injections on the Liver Concentration of Copper in Young Female Rats on a Purified Ration.

Rat Number	Treatment Basal Ration	Basal ration + 800 p.p.m. of Molybdenum as sodium molybdate	Basal ration + 800 p.p.m. of Molybdenum as sodium molybdate and bi-weekly intramuscular injections of 0.4 micrograms of Vitamin B_{12}
	Micrograms of Copper per gram of liver, dry weight.		
1	27.59	37.20	28.98
2	29.29	25.81	21.48
3	22.64	21.28	38.81
4	23.43	28.58	35.36
5	29.39	26.46	39.69
6	29.70	40.51	40.27
7	33.79	36.86	29.74
8	33.66	38.06	34.10
9	33.93	36.35	26.12
10	34.10	35.94	35.84
	29.75 ± 4.26[1]	32.71 ± 4.39	33.14 ± 6.25

[1] Standard deviation of the mean.

CONCLUSIONS

The results obtained in this experiment indicated that:

(1) Symmetrical alopecia and growth retardation are symptoms of molybdenum toxicity in young female rats.

(2) Vitamin B_{12} may act as an anti-alopecial factor for molybdenum induced alopecia.

(3) Vitamin B_{12} may be related to the growth depressing effect of molybdenum.

(4) Molybdenum had no effect on the liver copper concentration of young female rats under the conditions of this experiment.

(5) The injection of vitamin B_{12} had no effect on the concentration of copper in the liver of molybdenum supplemented rats.

(6) Rats appeared to make physiological adjustments with increased age for tolerance of a constant level of dietary molybdenum.

EXPERIMENT 4

The Effect of Adrenalectomy on Molybdenum Toxicity in Rats

Ralli and Graef (85, 86) have shown that either adrenalectomy or hypophysectomy will prevent the graying of the hair of black rats on a pantothenic acid deficient diet or will correct the condition once it has developed. Hundley and Ing (54) have extended these studies to copper deficient rats. They observed that adrenalectomy completely prevented development of gray hair in most copper deficient animals. Either adrenalectomy or hypophysectomy caused the partial or complete return of a normal appearing black hair coat in rats which had become gray on a copper deficient diet. Singer and Davis (96) have postulated an interrelationship between pantothenic acid and copper in the nutrition of the rat.

The findings of these investigators indicated that a metabolic relationship existed between copper, pantothenic acid and the adrenals. This experiment was designed to extend these studies on adrenal function to rats on a high dietary level of molybdenum.

[redacted]

Two groups of rats were used in this experiment in order to determine whether removal of the adrenals would prevent the appearance of molybdenum toxicity symptoms in young rats and whether it would alleviate the symptoms in mature rats.

Fifty four Piebald rats, averaging thirty days old were used in the first group. These rats were initially placed on the modified Ca-3 basal ration described in Experiment 2. Bilateral adrenalectomies were performed on twenty female and ten male rats. After a one week post-operative recovery period the survivors were divided according to sex and weight and placed on experimental rations along with unoperated controls of the same age. Twelve control and twelve adrenalectomized rats were maintained on the basal ration, while thirteen control and twelve adrenalectomized rats were placed on the basal ration supplemented with 2.06% grams of sodium molybdate.

The second group of rats used consisted of twenty two males that had been on the experimental rations described in the preceeding paragraph for twelve weeks. Five of the eleven rats on each ration were adrenalectomized. All of the rats were maintained on the experimental rations throughout the post-operative and subsequent experimental period.

One per cent sodium chloride, prepared with pyrex distilled water was given to all rats ad libitum from the time of the operation until one week before the termination of the experiment. During the last week of the experiment pyrex distilled water was substituted for the

one per cent sodium chloride and the adrenalectomized rats were checked for a loss in weight as an indication of complete adrenalectomy. All rats were weighed weekly. The rats in the first group were maintained on experimental rations for sixteen weeks and those in the second group for six weeks following the removal of the adrenals. Upon termination of the experiment all animals were sacrificed by exsanguination under ether anaesthesia. The livers were removed, weighed immediately and kept in a deep freeze until analyzed for copper. Post-mortem examinations were made to determine whether accessory or residual bits of adrenal tissue were present in the adrenalectomized rats, as a further check for complete adrenalectomy.

A number of the adrenalectomized male rats in Group 1 died within two weeks from the time of operation. The reason for the high mortality rate among the young male rats was not understood. A general thinning of the hair coat was observed in four of the adrenalectomized female rats on the basal ration within two weeks. This condition gradually improved and was no longer apparent by the sixth week. There was no evidence of alopecia in either the male or female adrenalectomized rats on the molybdenum supplemented ration, whereas, the unoperated controls developed characteristic symmetrical alopecia by the third week. The alopecia present in the molybdenum supplemented male rats in Group 2 at the time of adrenalectomy was alleviated by the fourth week after the operation, whereas, the unoperated controls still showed evidence of alopecia. Alopecia was not observed in the control and adrenalectomized male rats on the basal ration.

Table 13. presents a summary of the effect of adrenalectomy on the growth of normal and molybdenum supplemented rats. Reduced gains as a result of molybdenum supplementation were observed in the unoperated control animals. Adrenalectomy had no significant effect on the growth of female rats on the basal and molybdenum supplemented rations, however, it appeared to prevent growth retardation in the molybdenum supplemented male rats. Adrenalectomy caused an initial loss in weight in the mature rats in Group 2. The molybdenum supplemented rat recovered more quickly than those on the basal ration. However, at the end of

Table 13

Effect of Adrenalectomy on Growth of Normal and Molybdenum Supplemented Young Rats

Number of Animals	Sex	Treatment	Initial Weight (grams)	Weight gain (grams) 0-6 weeks	Weight gain (grams) 6-16 weeks
		[illegible]			
5	Male	Control	96	130 ± 21.5[1]	105 ± 15.1
6	Female	Control	77	95 ± 11.2	44 ± 13.7
7	Female	Adrenal-ectomized	75	93 ± 10.8	51 ± 12.5
		Basal Ration + 800 p.p.m. of Molybdenum as sodium molybdate			
7	Male	Control	72	87 ± 20.4	106 ± 28.1
3	Male	Adrenal-ectomized	98	135 ± 42.0	91 ± 36.0
6	Female	Control	83	82 ± 11.1	53 ± 9.9
8	Female	Adrenal-ectomized	67	75 ± 28.4	48 ± 10.5

[1]Standard deviation of the mean.

the experimental period there was little difference in the weight gains as a result of adrenalectomy or molybdenum supplementation.

Table 14. presents a summary of the effect of adrenalectomy on liver copper levels in rats before and after molybdenum supplementation. Adrenalectomy did not appear to affect the liver copper levels in either the rats on the basal or molybdenum supplemented rations. This observation is in agreement with that of Kozelka and Pedrero (62). They observed that bilateral adrenalectomy or thyroidectomy had no effect on copper levels in the kidney and liver of the albino rat. The liver copper levels observed in the rats on the basal ration were somewhat lower than those observed in Experiments 2 and 3 and suggested that the liver copper levels normally decreased with age. The molybdenum supplemented male rats also showed a decrease in liver copper levels when compared with those in Experiment 2, however, the copper levels in the female rats were similar to those observed in Experiments 2 and 3. Thus it appeared that sex was a factor in the relationship of molybdenum to copper metabolism.

There was no evidence of incomplete adrenalectomies in any of the rats used in this experiment.

Table 14

Effect of Adrenalectomy on Liver Copper Levels in Rats Before and After Molybdenum Supplementation[1]

Group	Number of Animals	Sex	Treatment	Micrograms of Copper per gram liver, dry weight
			[illegible]	
1	5	Male	Control	13.24 ± 3.87[2]
	6	Female	Control	15.15 ± 2.23
	7	Female	Adrenal-ectomized	17.17 ± 2.23
2	6	Male	Control	11.65 ± 3.46
	5	Male	Adrenal-ectomized	12.79 ± 4.79
			Basal Ration + 800 p.p.m. of Molybdenum as sodium molybdate	
1	6	Male	Control	13.97 ± 8.77
	3	Male	Adrenal-ectomized	12.92 ± 1.41
	6	Female	Control	27.65 ± 7.74
	5	Female	Adrenal-ectomized	22.59 ± 6.48
2	6	Male	Control	11.99 ± 4.90
	5	Male	Adrenal-ectomized	15.25 ± 5.19

[1]Group 1 was maintained on experimental rations for sixteen weeks after adrenalectomy and Group 2 was maintained on experimental rations twelve weeks prior to and six weeks after adrenalectomy.

[2]Standard deviation of the mean.

CONCLUSIONS

The results obtained in this experiment indicated that:

(1) Adrenalectomy prevented and alleviated alopecia due to a high dietary level of molybdenum.

(2) Early adrenalectomy did not affect the weight gain of young female rats on either a basal ration with or without molybdenum when given one per cent sodium chloride, however, there was an apparent increase in weight gain by young molybdenum supplemented male rats.

(3) Molybdenum induced alopecia may be related to adrenal function.

(4) Adrenalectomy had no apparent affect on liver copper levels.

(5) A high dietary level of molybdenum may interfere with a normal decrease in liver copper concentration with increased age.

EXPERIMENT 5

The Effect of a Low Dietary Level of Phosphorus on Molybdenum Toxicity in the Rat

Bone abnormalities in cattle associated with copper deficiency and molybdenum toxicity have been reported by Davis (25) and Cunningham (26). Shirley et al. (96, 97) have observed an altered excretion of phosphorus in phosphorus 32 studies with rats and cattle as a result of a high dietary level of molybdenum. These observations suggested that the level of phosphorus in the diet may be a factor in molybdenum toxicity. This experiment was carried out in order to determine the effect of a low dietary level of phosphorus on molybdenum toxicity in the rat.

Seventy Piebald rats, averaging thirty days old were randomized and lotted in the usual manner. The experimental rations contained two levels of phosphorus. The normal phosphorus (0.40 per cent) basal ration was the same as that described in Experiment 2. and the low phosphorus (0.18 per cent) basal ration was the same as the normal except for the omission of potassium phosphate (KH_2PO_4). Twenty rats were placed on each of these rations. Fifteen additional rats were placed on rations containing each level of phosphorus and supplemented with 2.064 grams of sodium molybdate per kilogram of ration. Pyrex distilled water was provided ad libitum for all groups. All animals were weighed weekly. The experiment was terminated after eighteen weeks at which time all animals were sacrificed by exsanguination under ether anaesthesia. The livers were removed and frozen. The livers of the molybdenum supplemented rats were analyzed for copper.

RESULTS AND DISCUSSION

The molybdenum supplemented male rats developed a moderate alopecia by the third week and were never completely free of it for the remainder of the experiment. The molybdenum supplemented female rats on the low phosphorus rations also developed alopecia early in the experiment but had fully recovered by the end of the sixth week, while the female rats on the normal phosphorus did not develop alopecia to an appreciable degree. There was no evidence of alopecia in the control groups. The male rats appeared to be more susceptible to molybdenum induced alopecia than were the females.

Table 15. presents a summary of the effect of molybdenum supplementation on the growth of rats on rations containing low and normal levels of phosphorus. The weight gains of the control rats during the first six weeks indicated that the low level of phosphorus had little effect; however, between the sixth and eighteenth week the male rats on the normal phosphorus ration gained considerably more than males on the low phosphorus ration. Molybdenum supplementation appeared to have a much greater effect on the growth of male rats on the low phosphorus ration during the first six weeks than it did on rats on the normal phosphorus ration. Comar et al. (21) have observed that the simultaneous oral administration of molybdenum and phosphorus resulted in a greater absorption of molybdenum. Thus it is suggested that the low phosphorus ration used, in this experiment, may have decreased absorption and maintained a higher intestinal level of molybdenum. If the toxic effect of

Table 15

The Effect of Molybdenum Supplementation on the Growth of Rats on Rations Containing Low and Normal Levels of Phosphorus

Number of Animals	Sex	Average initial weight (grams)	Average weight gain (grams) 0-6 weeks	6-18 weeks
		Low Phosphorus (0.18 per cent) Basal Ration		
10	Male	48	133 ± 8.3[1]	117 ± 17.1
10	Female	42	85 ± 9.1	61 ± 5.0
		Low Phosphorus (0.18 per cent) Basal Ration + 800 p.p.m. of Molybdenum as Sodium Molybdate		
8	Male	44	85 ± 21.6	152 ± 34.6
7	Female	42	73 ± 12.1	74 ± 12.6
		Normal Phosphorus (0.58 per cent) Basal Ration		
10	Male	51	139 ± 20.7	163 ± 29.6
10	Female	59	85 ± 18.8	57 ± 5.7
		Normal Phosphorus (0.58 per cent) Basal Ration + 800 p.p.m. of Molybdenum as Sodium Molybdate		
8	Male	40	114 ± 39.3	150 ± 36.5
7	Female	46	103 ± 19.3	61 ± 10.6

[1] Standard deviation of the mean.

molybdenum is exerted in the intestines, a higher intestinal concentration, such as might result from a low phosphorus intake could account for the poorer initial gains made by the rats on the molybdenum supplemented low phosphorus ration. The normal gains made by the molybdenum supplemented rats after the sixth week indicated that the rat was able to make a physiological adjustment at either a low or normal level of phosphorus.

Table 16. presents the effect of low and normal phosphorus intakes on liver copper in molybdenum supplemented rats. The results here are essentially the same as those observed in Experiment 4. and indicated that the phosphorus levels used in this experiment did not influence the liver copper concentrations.

Table 16

The Effect of Low and Normal Phosphorus Intakes on Liver Copper in Molybdenum Supplemented Rats

Rat Number	Sex	Micrograms of Copper per gram liver, dry weight	Rat Number	Sex	Micrograms of copper per gram liver, dry weight
		Low Phosphorus (0.16 per cent) Basal Ration + 300 p.p.m. of Molybdenum as Sodium Molybdate			
1	Male	15.05	1	Female	24.57
2	Male	20.19	2	Female	80.64
3	Male	14.68	3	Female	50.16
4	Male	12.86	4	Female	24.72
5	Male	8.93	5	Female	20.88
6	Male	8.81	6	Female	37.86
7	Male	21.12	7	Female	22.29
8	Male	16.57			
	Average	14.77 ± 4.58[1]			38.73 ± 11.14
		Normal Phosphorus (0.52 per cent) Basal Ration + 300 p.p.m. Molybdenum as Sodium Molybdate			
1	Male	11.14	1	Female	51.75
2	Male	15.67	2	Female	27.51
3	Male	9.88	3	Female	34.65
4	Male	11.47	4	Female	20.52
5	Male	14.68	5	Female	21.66
6	Male	9.79	6	Female	28.08
7	Male	10.38	7	Female	24.36
8	Male	18.64			
	Average	12.63 ± 3.38			29.79 ± 10.77

[1]Standard deviation of the mean.

CONCLUSIONS

The results obtained in this experiment indicated that:

(1) Molybdenum had a greater initial toxicity for rats on a low phosphorus ration than for rats on a normal phosphorus ration.

(2) Initial molybdenum toxicity may be related to its concentration in the lower part of the intestinal tract.

(3) Reducing the phosphorus level in the ration from 0.40 to 0.18 per cent did not affect the liver copper level in molybdenum supplemented rat.

EXPERIMENT 6

Molybdenum Metabolism and Interactions in Beef Calves

Molybdenum toxicity in cattle constitutes a problem of major importance in many areas particularly on reclaimed peat and muck soils. In most instances copper therapy (39, 40) will alleviate the toxicity symptoms. In some cases, however, the further addition of cobalt is necessary (13). Factors other than copper, capable of alleviating molybdenum toxicity symptoms in sheep, have been shown to be present in lucerne hay (30).

Failure of proper bone formation in cattle has been observed in copper deficient areas (26, 28). The bone abnormalities are characterised by a rachitic-like condition and a high incidence of fractures. In older animals osteomalacia was observed. By giving molybdenum supplements to young heifers on copper deficient pastures the incidence of bone fractures was increased over that observed in unsupplemented heifers (28). Thus it would appear that molybdenum aggravated the condition. Molybdenum supplementation of steers in dry lot has been shown to cause an alteration in the pathway of phosphorus excretion (95).

The purpose of this experiment was to study in young calves the influence of a moderate excess of dietary molybdenum on:

(1) Growth

(2) B vitamin levels in the blood and liver

(3) Liver copper concentration.

(4) Calcium and phosphorus balance.

(5) The behavior of intravenously administered radiocalcium.

(6) The behavior of intravenously administered radiomolybdenum.

Eight Hereford calves averaging five months of age were obtained from the UT-AEC Agricultural Research Program herd. These calves had been reared under average farm conditions and were on pasture at the time of selection. They were placed in stalls equipped with stanchions and individually fed a ration of calf meal[1] and orchard grass hay during a ten day pre-experimental period. Water was provided ad libitum. At the end of this period all of the calves were consuming four pounds of calf meal and four pounds of orchard grass hay per day. Chemical analysis expressed on an air dry basis indicated that the calf meal contained 1.47 per cent calcium, 0.92 per cent phosphorus and 36.9 p.p.m. of copper; the orchard grass hay contained 0.24 per cent calcium, 0.27 per cent phosphorus and 12.1 p.p.m. of copper.

At the beginning of the experimental period the calves were paired according to age and weight. Pair feeding was employed throughout the experimental period. One calf from each pair was fed the basal ration of calf meal and orchard grass hay and the other calf was fed the basal ration supplemented with sodium molybdate to provide a level of 200 p.p.m. of molybdenum. The sodium molybdate was incorporated in the calf meal using a Twin Spiral mixer of one half ton capacity manu-

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[1] Purina Calf Chow, Ralston Purina Company, St. Louis, Missouri.
Guaranteed analysis: crude protein not less than 27.0 per cent
crude fat not less than 2.5 per cent
crude fiber not more than 5.0 per cent
nitrogen free extract not less than 42.0 per cent

factured by the Prater Pulverizer Company, Chicago, Illinois.

Blood samples were taken from three pairs of calves after twenty five days on experimental rations. At this time two of the calves receiving the molybdenum supplemented ration were scouring profusely. Two milliliters of blood from each sample were digested enzymatically by a slight modification of the method of Cheldelin et al. (15) in which mylase-P was substituted for takadiastase. Microbiological assays for niacin, pantothenic acid, riboflavin and vitamin B_{12} were run on aliquots of the enzymatically digested blood samples. Corrections were made for the B vitamin content of the enzyme suspension added to each sample of blood.

Calcium 45 studies were conducted on three pairs of calves. Pairs of calves were placed in metabolism units as described by Hansard (46) after periods of forty, forty-two and fifty-three days on experimental rations. Twenty-four hour adjustment periods were allowed. Following this each calf was given approximately 1.23 millicuries of radiocalcium intravenously using the technique of Hansard et al. (47). Blood samples were taken at specified intervals of time after injection. Concurrent ten day calcium, phosphorus and radiocalcium balance studies were started at the time of dosing, with the exception of the first pair of calves (numbers 220 and 234), which were sacrificed twenty-four hours after isotope administration. Quantitative collections of feces and urine were made and daily samples of each were taken for analysis during the balance periods. Pairs of calves were slaughtered and samples of selected tissues, bones and blood obtained, according to the

procedure of Hazzard et al. (48), one day, ten days and eighty three days after injection. Analyses for total calcium and radiocalcium were carried out as described by Comar et al. (23). Phosphorus was determined by the method of Fiske and SubbaRow (41).

Molybdenum 99 studies were conducted with one pair of calves. After forty days on experimental rations they were transferred to metabolism units and following an initial adjustment period were given approximately 4.43 millicuries of radiomolybdenum intravenously. Blood samples were taken at periodic intervals after dosing. The calves were slaughtered and samples of selected tissues, bones and blood obtained. Quantitative collections of feces and urine were made and samples of each were taken for radiochemical determination. The same procedures for handling, dosing and slaughtering the calves used in the calcium 45 study were employed. Samples were prepared for radiomolybdenum determinations by wet ashing with concentrated nitric acid as described by Comar et al. (21).

Autoradiograms were prepared from sections of the metatarsus bone of each calf according to the method of Lota et al. (68). Liver samples obtained at the time of sacrifice were analysed for Vitamin B_{12} and copper. The non-enzymatic method of digestion described by Schied et al. (93), was used to liberate Vitamin B_{12}. Tissue samples for histological examination taken at the time of sacrifice were placed in sample bottles containing ten per cent formalin solution and stored in a refrigerator until sectioned and stained.

RESULTS AND DISCUSSION

Typical clinical symptoms of molybdenum toxicity were produced in two of four calves receiving a dietary level of 200 p.p.m. of molybdenum as sodium molybdate. The first apparent symptom was a severe diarrhea which developed by the end of the second week. The diarrhea persisted in its severity for the remainder of the first month, then moderated and appeared intermittently throughout the remainder of the experiment. The failure to produce diarrhea in all of the calves receiving molybdenum indicated that the level may have been near the critical value for calves of that age under the conditions of the experiment. The relatively high level of copper, 24.5 p.p.m. in the basal ration may also have been a contributing factor. Henson (50) in feeding the same level of molybdenum for a prolonged period to three year old Jersey steers failed to produce marked symptoms of toxicity. Thomas and Moss (112) administered considerably higher levels of molybdenum to male Holstein calves and produced a mild diarrhea of short duration.

Varying degrees of lameness were produced in the molybdenum supplemented calves. Although locomotion did not appear to be impaired the calves walked with a stiff gait as if in pain. There was no apparent swelling or outward indication of joint abnormalities. A slight beading of the ribs was observed on calf 224 near the end of the experiment. Similar observations have been made on cattle grazing on molybdenum-toxic pastures (14, 26, 28) and in calves receiving molybdenum in dry lot (112).

Hair coat changes were apparent after the fifth week. The normal reddish brown color of the Hereford calves gradually faded and took on a yellow brown cast. In addition to color change there was a loss of luster and thinning of the hair coat. These changes are shown in Figure 2 which contrasts a molybdenum supplemented calf with its pair mate after 120 days on experiment.

The effect of molybdenum on weight gain is presented in Table 17. Calves 234 and 224 show a marked depression in daily gains for the first forty days of the experiment. There was no apparent loss of appetite except for a reduced hay consumption during the period spent in metabolism units. Growth depression as a result of an excess of molybdenum in the diet has been widely observed (14, 26, 28, 36). Calves 222 and 226 showed more rapid daily gains than the other calves. This may in part be due to nutritional status and age at the time placed on experiment. Molybdenum in the diet did not appear to retard the growth of calf 226. The rate of gain for calf 224 increased considerably during the last eighty three days of the experiment. There was no change in the molybdenum level in the total ration during this period. The increased rate of gain shown by this calf may be attributable to a reduced incidence of diarrhea and to physiological adjustment.

Table 18 presents the results of microbiological assays for B vitamins run on blood samples taken during the fourth week of the experiment. The results indicated that molybdenum had little effect on the level of nicotinic acid, pantothenic acid and riboflavin in the blood; however, there was a lowered level of vitamin B_{12} activity. Experiments

Figure 2. Control (page 84) and molybdenum (page 85) supplemented calves after 120 days on experiment. Note the lack of alertness and rough thin hair coat of the molybdenum supplemented calf.

Figure 2. (Continued) Control (page 84) and molybdenum (page 85) supplemented calves after 120 days on experiment. Note the lack of alertness and rough thin hair coat of the molybdenum supplemented calf.

Table 17.

Effect of High Dietary Level of Molybdenum on Weight Gain by Pair Fed Beef Calves (Expressed in pounds)

Pair Number	Animal Number	Initial Age (months)	Sex	Initial Weight	Weight After 40 Days	Daily Gain (40 Days)	Weight After 125 Days	Daily Gain (125 Days)
				Basal Ration				
1	220	4.5	Male	306	360	1.35		
2	213	5.0	Male	372	432	1.50		
3	231	4.0	Steer	334	388	1.35	683	1.19
4	222	6.0	Steer	416	488	1.80		
				Basal Ration + 200 p.p.m. Molybdenum				
1	234	3.5	Female	272	306	0.85		
2	217	4.5	Steer	362	416	1.35		
3	224	4.5	Steer	328	350	0.55	440	0.90
4	228	6.0	Steer	480	570	2.25		

Table 18

Comparison of the "B Vitamin" Values of Whole Blood of Beef Calves in Early Stage[1] of Molybdenum Toxicity with that of Normal Calves

Pair Number	Animal Number	Sex	Pantothenic Acid	Riboflavin	Nicotinic Acid	Vitamin B_{12} Activity
			micrograms per 100 milliliters			millimicrograms per 100 milliliters
			Basal Ration			
1	220	Male	34	62	525	40
2	213	Male	35	73	450	35
3	231	Steer	38	78	521	38
			Basal Ration + 200 p.p.m. Molybdenum			
1	234	Female	25	58	318	22
2	217	Steer	28	54	506	31
3	224	Steer	34	75	605	20

[1] Blood samples taken 25 days after initiation of experiment.

conducted by Ray et al. (67) showed that a cobalt deficiency in sheep did not materially affect the level of pantothenic acid and riboflavin in the blood but did cause a reduction in the nicotinic acid level. Hoekstra et al. (52) in similar studies with sheep showed that a cobalt deficiency caused a slight reduction in the riboflavin and nicotinic acid content of rumen ingesta and a marked reduction of vitamin B_{12} activity in the blood.

Table 19 presents the vitamin B_{12} data of the livers before and after alkali treatment for the two groups. The difference in the two values gives an approximation of the "true" vitamin B_{12} activity. The concentration of "true" vitamin B_{12} activity of the liver also appears to be affected by molybdenum. Three of the calves receiving supplemental molybdenum showed a reduction in "true" vitamin B_{12} concentration in the liver, while the fourth showed a marked depletion. The concentration of alkali stable vitamin B_{12} activity did not appear to be affected by molybdenum. The blood levels of total vitamin B_{12} activity in calves 22k and 23k were similar after twenty five days on experiment, however, the liver concentrations at the time of slaughter were quite different. This may be due to physiological adjustment during the extended period calf 22k was kept on experiment. The levels of vitamin B_{12} activity observed in the blood and liver of molybdenum supplemented calves suggests that molybdenum may interfere with the intermediary metabolism of cobalt.

A reduction in liver copper concentration was observed in the molybdenum supplemented calves used in this experiment, (see Table 20).

Table 19

Effect of Dietary Molybdenum Upon the Vitamin B_{12} Content of Calf Liver

Pair Number	Animal Number	Sex	Days on Experiment	Vitamin B_{12} Activity	Alkali Stable Vitamin B_{12} Activity
				micrograms per 100 grams fresh weight	
			[illegible]		
1	220	Male	40	62	2.5
2	213	Male	52	60	3.4
3	231	Steer	125	70	2.8
4	222	Steer	40	80	3.1
			Basal Ration + 300 p.p.m. Molybdenum		
1	234	Female	40	8	2.7
2	217	Steer	52	33	3.0
3	224	Steer	125	60	2.3
4	228	Steer	40	30	2.6

[illegible]

Effect of Dietary Molybdenum Upon the Liver Copper Content of Beef Calves

Pair Number	Animal Number	Sex	Days on Experiment	Micrograms of copper per gram dry weight	Milligrams of copper total liver
			Basal Ration		
1	220	Male	40	224.2	190
2	213	Male	52	277.5	215
3	231	Steer	125	128.2	114
4	222	Steer	40	195.0	158
			Basal Ration + 200 p.p.m. Molybdenum		
1	234	Female	40	118.8	102
2	217	Steer	52	173.0	131
3	224	Steer	125	154.3	127
4	226	Steer	40	177.2	125

In no case was the lowering of the concentration of liver copper excessive. Similar results were obtained by Hanson (50) in studies with Jersey steers. The relatively high level of copper present in the basal ration may account for the small reduction in liver copper concentration observed. Calf 234 did not show a marked depletion of liver copper as might have been expected if molybdenum was interfering with cobalt metabolism. The level of cobalt in the basal ration was not determined, however, a cobalt carbonate supplement had been added to the calf meal by the manufacturer. Kidder et al. (61) have shown that the addition of cobalt to the mineral mixture of steers grazing on pastures containing an adequate level of copper allowed the assimilation of the copper in the forage, whereas the copper reserves were depleted in the livers of steers not receiving cobalt.

Tables 21, 22 and 23 present values for the excretion of calcium, phosphorus and radiocalcium during ten day balance periods starting at the time of intravenous administration of radiocalcium. An average of sixty nine per cent of the dietary calcium and approximately seventy three per cent of the dietary phosphorus was excreted by both control and molybdenum supplemented calves. Molybdenum supplementation did not cause an apparent alteration in the pathway of phosphorus excretion such as observed by Shirley et al. (95) in phosphorus 32 studies with molybdenum supplemented Jersey steers. This would indicate that an altered phosphorus metabolism is a latent effect of excess molybdenum in the diet. The average radiocalcium excretion was 8.9 per cent of the administered dose for the control and 8.6 per cent for the molybdenum

Table 21

Effect of High Dietary Level of Molybdenum on Calcium Balance[1] in Beef Steers

Animal Number	Age (months)	Weight (pounds)	Average Daily Ca Intake (grams)	Average Daily Ca in Feces (grams)	Balance	Per cent Ca Retained
			Basal Ration			
213	6.5	459	31.0	22.3	+ 8.7	72
231	5.5	368	30.5	20.0	+ 9.5	66
			Basal Ration + 200 p.p.m. Molybdenum			
227	6.5	436	29.5	18.5	+11.0	63
224	6.0	350	30.3	22.6	+ 7.7	75

[1]Balance trial run for 10 day period.

[redacted]

Effect of High Dietary Level of Molybdenum on Phosphorus Balance[1] in Beef Steers

Animal Number	Age (Months)	Weight (Pounds)	Average Daily P Intake (grams)	Average Daily P in Feces (grams)	Average Daily P in Urine (grams)	Balance	Per cent P Excreted
				Basal Ration			
213	6.5	458	21.5	10.8	5.1	+ 5.6	73.9
231	5.5	388	21.0	9.6	5.6	+ 5.8	72.4
				Basal Ration + 200 p.p.m. Molybdenum			
217	6.5	436	19.9	10.2	3.7	+ 6.0	69.8
224	6.0	350	20.8	12.5	3.9	+ 4.4	78.8

[1] Balance trial run for 10 day period.

Table 23

Effect of High Dietary Level of Molybdenum on Excretion of Intravenously Administered Ca^{45} by Beef Steers (Expressed as per cent of dose per day)

Treatment	Basal Ration		Basal Ration + 200 p.p.m. molybdenum	
Animal Number	213		217	
Age and Weight (pounds)	6.5 months, 458		6.5 months, 436	
Time (days)	Feces	Urine	Feces	Urine
1	4.83	0.027	3.76	0.050
2	2.10	0.006	1.70	0.009
3	1.19	0.008	0.72	0.007
4	1.01	0.002	0.83	0.008
5	0.36	0.004	0.56	0.005
6	0.24	0.003	0.43	0.002
7	0.20	0.002	0.48	0.001
8	0.18	0.001	0.25	0.003
9	0.18	0.001	0.16	0.004
10	0.18	0.001	0.33	0.008
Total	10.47	0.055	9.22	0.090
Animal Number	233		234	
Age and Weight (pounds)	5.5 months, 388		6.0 months, 350	
Time (days)	Feces	Urine	Feces	Urine
1	3.75	0.017	3.13	0.055
2	1.27	0.007	1.59	0.015
3	0.64	0.006	0.95	0.012
4	0.37	0.006	0.53	0.007
5	0.29	0.006	0.37	0.003
6	0.27	0.004	0.30	0.008
7	0.22	0.005	0.20	0.004
8	0.16	0.007	0.21	0.002
9	0.15	0.013	0.21	0.003
10	0.14	0.006	0.21	0.003
Total	7.26	0.077	7.70	0.115

supplemented calves during the ten day period following dosing. Maximum excretion of radiocalcium occurred during the first twenty four hours.

Radiocalcium Studies: Table 24 shows the amount of radiocalcium in the blood plasma as a function of time after intravenous injection. The results represent averages of two control and two molybdenum supplemented animals. Total blood was calculated as 5.7 per cent of body weight (49). The initial disappearance rate of radiocalcium from the plasma appeared to be slightly more rapid in the control calves than in the molybdenum supplemented calves. This was in agreement with observation of a greater fecal excretion during the first twenty four hours and a greater laydown of radiocalcium in the metatarsus of the control calves. One minute after injection an average of 28.7 per cent of the administered dose was present in the plasma while at four hours an average of only 4.7 per cent remained. This level was further reduced to an average of 0.85 per cent twenty four hours after injection. Excretion data already presented indicated that retention of radiocalcium was approximately ninety per cent of the administered dose and analytical values showed that most of the retained calcium 45 ultimately was found in bone. Thus it is apparent that the disappearance of radiocalcium from the blood is primarily due to a rapid accumulation in the skeleton.

Table 25 demonstrates the effect of molybdenum on the distribution of radiocalcium in selected tissues of beef calves at intervals of one, ten and eighty three days after intravenous injection. Concentration values are expressed as per cent of the dose times 10^5 per gram fresh

Table 24

Effect of High Dietary Level of Molybdenum on the Disappearance of Calcium 45 from the Blood[1] of Beef Steers Following Intravenous Administration

Treatment		Basal Ration	Basal Ration + 200 p.p.m. Molybdenum
Time after Administration			
Hours	Minutes	Per cent dose in plasma	Per cent dose in plasma
	1	37.4	40.0
	3	25.3	32.4
	5	22.3	25.5
	7.5	18.3	20.2
	10	23.2	20.0
	15	15.1	12.1
	45	9.7	9.8
1		8.4	6.9
2		5.6	4.7
4		0.69	1.1
6		0.34	0.74
72		0.49	0.39
96		0.28	0.39
144		0.16	0.24
168		0.11	0.21

[1]Calculations based on blood volume of 5.7 per cent of body weight.

Table 25

Effect of High Dietary Level of Molybdenum on the Distribution of Calcium 45 in Selected Tissues[1] of Beef Calves Following Intravenous Administration

Treatment	Basal Ration		Basal Ration + 200 p.p.m. Molybdenum	
Tissue	Per cent dose x 10^3 per gram fresh weight	Specific[2] Activity	Per cent dose x 10^3 per gram fresh weight	Specific Activity
		1 Day		
Blood	18.55	210.8	15.23	163.8
Liver	15.92	217.9	13.25	194.9
Kidney	24.70	164.6	20.88	158.2
Spleen	17.24	205.2	13.52	155.4
Gastrocnemius Muscle	8.62	146.1	8.18	131.9
Metatarsus Shaft	148.4	.68	142.2	.64
Metatarsus Epiphysis	331.2	2.72	257.2	2.60
		10 Days		
Blood	.423	8.39	.561	7.36
Liver	.614	9.67	.385	7.63
Kidney	.613	8.63	.853	9.60
Spleen	.459	9.58	.672	10.35
Gastrocnemius Muscle	.262	8.76	.216	6.70
Metatarsus Shaft	264.4	1.25	210.3	0.93
Metatarsus Epiphysis	392.6	3.18	248.4	2.20
Rib Shaft	345.9	2.10	345.0	2.10
Rib Sternal End	967.9	11.80	962.1	10.94

Table 25 (Continued)

Effect of High Dietary Level of Molybdenum on the Distribution of Calcium 45 in Selected Tissues[1] of Beef Calves Following Intravenous Administration

Treatment	Basal Ration		Basal Ration + 200 p.p.m. Molybdenum	
Tissue	Per cent dose x 10^5 per gram fresh weight	Specific[2] Activity	Per cent dose x 10^5 per gram fresh weight	Specific Activity
[illegible]				
Blood	.377	4.63	.799	8.47
Liver	.427	3.30	.654	7.92
Kidney	.820	4.15	1.206	8.84
Spleen	.438	4.11	.450	4.53
[illegible]				
Muscle	.296	4.90	.551	8.56
Metatarsus Shaft	135.9	.68	177.4	.89
Metatarsus Epiphysis	240.6	2.31	220.8	1.74
Rib Shaft	259.1	1.76	378.6	2.52
Rib Sternal End	120.0	1.53	125.1	1.48

[1]Calculations standardized to body weight of 500 pounds.

[2]Specific activity expressed as per cent of dose x 10^5 per gram of calcium.

weight and specific activity values are expressed as per cent of the dose times 10^5 per milligram of calcium. The specific activity values for the soft tissues indicated that circulating radiocalcium rapidly reached equilibrium between the soft tissues and the plasma and remained in equilibrium. The concentration of radiocalcium in the bones ranged from less than one hundred one day after injection, to several hundred times that in the soft tissues ten days after injection. This increased concentration in the bone with time indicated an accumulation of calcium 45.

Autoradiographic observations indicated that molybdenum did not appear to have any affect on the rate of accumulation of radiocalcium in the metatarsus during the first ten days following injection. Radiochemical determinations, however, indicated that molybdenum caused a slight decrease in the accumulation of radiocalcium in the metatarsus, but there was no evidence of such a decrease in the rib shaft or rib sternal end. The ratio of the specific activity in the gastrocnemius muscle to that in the metatarsus shaft after radiocalcium equilibrium was attained provided an index to the relative state of calcium metabolism. Ten days after dosing the control calf had an index of 7.0 and the molybdenum supplemented calf 7.2. Eighty three days after dosing the control calf had an index of 7.2 and the molybdenum supplemented calf 9.6. Thus it is suggestive that resorption from the bone occurred at a greater rate from the molybdenum supplemented calf. This increased rate of resorption was reflected by a higher concentration of radiocalcium in the blood and soft tissues. From these observations it would

appear that molybdenum does have a latent effect on calcium metabolism that may be the consequence or cause of an altered phosphorus metabolism.

Autoradiographic studies essentially reflected the results of the radiochemical determinations. There was no apparent difference in the laydown pattern of radiocalcium in the metatarsus ten days after intravenous administration. Bone growth did not appear to be affected by molybdenum. Autoradiograms prepared from sagittal sections of metatarsi taken eighty three days after dosing are presented in Figure 3. The black spots indicate Ca^{45} and the darker areas the site of original deposition. These autoradiograms essentially confirm the increased rate of resorption of radiocalcium indicated by the radiochemical determinations.

Table 26 presents the calcium and phosphorus values of selected tissues in the calves used in this experiment. Statistical analysis of these results were not applied because of the limited number of animals used. However, the observations on the behavior of intravenously administered radiocalcium in normal calves are essentially in agreement with earlier studies reported on by Hansard et al. (48).

The disappearance of intravenously administered radiomolybdenum and the effects of stable molybdenum thereon are presented in Table 27. Molybdenum supplementation apparently caused a slight decrease in the disappearance rate. This was probably the result of a higher blood and tissue level of stable molybdenum.

Radiomolybdenum was removed from the plasma at about the same initial rate as was radiocalcium. This means that the penetration into

Figure 3. Autoradiograms of metatarsi from control (right) and molybdenum supplemented (left) steers sacrificed eighty three days after intravenous administration of Ca^{45}. Note that in the molybdenum animal there was a greater resorption of Ca^{45} from the site of original epiphyseal laydown, however, bone growth did not appear to be affected.

Table 26

Effect of High Dietary Level of Molybdenum on the Calcium and Phosphorus Content of Selected Tissues from Beef Calves

Treatment	Basal Ration			Basal Ration + 200 p.p.m. Molybdenum		
Days on Experiment	40	52	125	40	52	125
Age of Animal (months)	6.0	6.5	5.5	5.0	6.5	6.0
Animal Number	220	213	231	234	217	224
Tissue	Milligrams calcium per gram tissue, fresh weight					
Blood	.088	.074	.082	.093	.076	.094
Liver	.073	.064	.129	.083	.050	.068
Kidney	.150	.071	.197	.132	.089	.136
Spleen	.084	.048	.104	.087	.065	.099
Gastrocnemius Muscle	.059	.030	.061	.065	.032	.062
Metatarsus Shaft	218.	228.	200.	221.	227.	199.
Metatarsus Epiphysis	122.	124.	104.	127.	113.	99.
Rib Shaft	*	165.	147.	*	159.	150.
Rib Sternal End	*	82.	79.	*	88.	85.
Tissue	Milligrams phosphorus per gram tissue, fresh weight					
Blood	0.57	0.57	0.43	0.55	0.56	0.40
Liver	2.67	2.47	3.21	2.84	3.38	3.58
Kidney	3.18	2.84	3.44	2.56	2.92	3.25
Spleen	3.16	2.54	3.46	2.38	2.89	3.65
Gastrocnemius Muscle	2.04	1.63	2.14	2.25	1.74	2.44
Metatarsus Shaft	89.	107.	138.	88.	102.	129.
Metatarsus Epiphysis	42.	50.	73.	39.	54.	70.
Rib Shaft	*	78.	105.	*	64.	116.
Rib Sternal End	*	42.	58.	*	46.	67.

* Values not determined.

Table 27

Effect of High Dietary Level of Molybdenum on the Tissue Distribution[1] of Molybdenum 99 in Beef Steers Following Intravenous Administration

Treatment	Basal Ration	Basal Ration + 200 p.p.m. Molybdenum
Animal Number	222	228
Age and Weight (pounds)	6.5 months, 488	6.5 months, 570
Actual dosage, grams of MoO_3	2.6	2.6
Sacrificed after (hours)	24	24
Tissue	Per cent of dose x 10^3 per gram of tissue fresh weight	
Adrenal	15.1	25.2
Pituitary	10.34	11.9
Blood	15.1	18.6
Liver	12.0	14.0
Kidney	26.9	18.8
Gastrocnemius Muscle	2.2	2.1
Loin Muscle	15.1	18.6
Pancreas	9.0	*
Spleen	12.3	10.6
Small Intestines	18.8	8.4
Large Intestines	9.0	8.5
Intestinal Lymph Node	15.8	12.5
Rumen	7.0	8.8
Reticulum	*	9.6
Omasum	7.7	21.8
Abomasum	11.0	*
Mandible	39.8	45.2
Metatarsus Shaft	27.8	18.7
Metatarsus Epiphysis	14.7	15.0
Rib Shaft	31.0	31.8
Rib Sternal End	26.0	31.1
Incisors	11.6	13.2

[1]Calculations standardized to a body weight of 500 pounds.

*Samples lost.

the extracellular fluids was probably the same for both elements. However after a few hours the comparative disappearance rate for the Mo^{99} lagged behind that for Ca^{45} which was probably a reflection of the greater avidity of bone for the labeled calcium since there was apparently rapid equilibrium between plasma and soft tissues.

The retarded disappearance of molybdenum may be due to the failure of a high accumulation in the bone such as observed for radiocalcium. The soft tissue accumulation for the two isotopes twenty four hours after dosing was comparable.

Radiochemical determinations indicated that intravenously administered radiomolybdenum was rapidly excreted with 52.4 and 60.4 per cent of the dose excreted in the urine of the control and molybdenum supplemented calves respectively, during the twenty four hour period following injection. The fecal excretion during this period was less than 1.0 per cent. The observed Mo^{99} excretion values were considerably higher than those reported by Comar et al. (21). It was apparent therefore that blood molybdenum was primarily excreted via the kidneys.

The distribution of intravenously administered radiomolybdenum in selected tissues of pair fed calves sacrificed twenty four hours after dosing is presented in Table 28. These data are essentially in agreement with earlier studies reported on by Comar et al. (21). The low concentration of radiomolybdenum present in the metatarsi, together with its short half life, prevented the development of satisfactory autoradiograms.

Histological examinations of sections of the adrenals, pituitary

and the sternal end of the rib failed to reveal any pathological changes as a result of molybdenum supplementation.

Table 28

Effect of High Dietary Level of Molybdenum on the Disappearance of Molybdenum 99 from the Plasma and Whole Blood[1] of Beef Steers Following Intravenous Administration (Expressed as per cent of dose in total plasma or blood)

Treatment		Basal Ration		Basal Ration + 200 p.p.m. Molybdenum	
Time after Administration					
Hours	Minutes	Plasma	Blood	Plasma	Blood
	1	34.1	39.5	41.8	44.8
	2	31.0	34.7	39.3	42.9
	3	33.1	35.0	35.8	40.2
	5	28.5	31.0	34.2	36.1
	10	25.3	27.9	29.2	31.2
	20	21.2	24.5	25.2	26.5
	45	17.3	18.9	19.9	21.2
1		16.3	16.7	18.2	19.4
4		9.0	10.0	10.1	10.5
24		2.6	2.8	2.8	3.0

[1] Calculations based on blood volume of 5.7 per cent of body weight.

CONCLUSIONS

The results obtained in this experiment indicate that:

(1) Molybdenum interferred with the growth of young Hereford calves.

(2) Nutritional status and age may be factors in determining the relative toxicity for a given level of molybdenum in the diet.

(3) Molybdenum may interfere with the intermediary metabolism of cobalt. This was reflected by a reduced liver concentration of vitamin B_{12}.

(4) Molybdenum apparently does not affect the level of nicotinic acid, pantothenic acid and riboflavin in the blood.

(5) An excess of molybdenum in the diet containing approximately three times the normal requirement for copper resulted in a lowering of the liver copper concentration.

(6) Molybdenum appears to have a latent effect on calcium metabolism, as demonstrated by increased de-mineralization.

(7) Dietary molybdenum appears to have little effect on the distribution and excretion of intravenously administered radiomolybdenum.

GENERAL DISCUSSION

The physiological role of molybdenum in animal nutrition has been of interest since this element was first recognized as being involved in copper metabolism. Experiments with farm and laboratory animals have established certain relationships concerning the physiological role of this mineral. The relative toxicity of ingested molybdenum varies greatly with species. Monogastric animals in general have been shown to have a much higher tolerance for molybdenum than ruminating animals. This may be the consequence of basic anatomical and physiological differences or differences in dietary requirements particularly with respect to members of the vitamin B complex. In this study many of the established effects and relationships of molybdenum in the nutrition of rats and cattle have been confirmed. However, certain additional observations will be discussed.

A symmetrical alopecia was consistently observed in weanling rats on a purified ration containing 5 p.p.m. of copper and 800 p.p.m. of molybdenum. On these rations alopecia usually developed between the second and third week and reached maximum severity by the fifth week after which there was a gradual diminution. Male rats were affected to a greater degree than were female rats. Alopecia <u>per se</u> was not observed in molybdenum supplemented calves, however, there was a general thinning of the hair coat, lack of luster and a change from a reddish brown to a yellow brown color. The syndrome was prevented in young rats by bi-weekly intramuscular injections of 0.4 micrograms of vitamin

B_{12} and by adrenalectomy. Adrenalectomy also alleviated molybdenum induced alopecia in mature male rats. These results suggested that molybdenum may interfere with the central mechanisms involved in the maintenance of normal levels of adrenal cortical hormones and that alopecia and achromotrichia may be a consequence of a high level of cortical hormones. A relationship between pantothenic acid, vitamin B_{12}, copper and the adrenals has been reported by others (85, 86, 118, 53). Apparently, deficiencies of either pantothenic acid or copper interfere with the normal degradation of cortical hormones. Abnormally high levels of cortical hormones have been shown to cause a decrease in normal pigmentation and growth of hair (7).

Since alopecia and achromotrichia are associated with high levels of cortical hormones in the rat, it is possible that the similar outward symptoms of copper deficiency, pantothenic acid deficiency and molybdenum toxicity are manifestations of the same basic disturbance. The ability of intramuscularly administered vitamin B_{12} to prevent the development of alopecia in molybdenum supplemented rats suggested that this vitamin may also play a role in the degradation and elimination of cortical hormones or may be related to the absorption and excretion of molybdenum. Under the conditions of this experiment the cortical hormone concentration did not reach a level high enough to cause changes in bone structure such as those observed in pantothenic acid deficient rats (81) and as a result of injecting high levels of cortisone (43). The failure of the cortical hormones to reach a level high enough to cause changes in the bone structure may have been due to a depressing

effect on the hypophysis with a corresponding reduction in adrenal stimulus.

In rats, molybdenum supplementation resulted in a marked depression of growth until the animals were approximately ten weeks old after which the same level of molybdenum in the diet had no apparent effect on growth. The rats resumed normal growth at about the time that they reached sexual maturity. Thus it was suggested that changes in endocrine balance normally occuring at this time may have been responsible for the reduced toxicity. Vitamin B_{12} injections gave a partial alleviation of the growth retarding effect of molybdenum, however, this may have been an independent effect of vitamin B_{12}. There is also the possibility that greater alleviation might have been obtained if an equivalent amount of vitamin B_{12} had been administered orally. Liver weight to body weight ratio studies gave no indication of an altered vitamin B_{12} status as a result of molybdenum supplementation. Although this technique has been used by others (117, 127) it may not be a reliable index of vitamin B_{12} status under the conditions of this study.

In young rats molybdenum supplementation resulted in a higher concentration of copper in the liver but had little effect on the total amount of copper present since these rats had smaller livers. Thus it was suggested that the higher levels observed were the result of a failure of utilization rather than an accumulation. The physiological availability of copper under these conditions has been questioned (64, 72). Comar et al. (21) were able to prevent the appearance of toxicity symptoms in young rats receiving 80 p.p.m. of

molybdenum in the diet by increasing the copper level. However, studies by Kulwich (64) indicated that when molybdenum was fed at a level of 1000 p.p.m. in the ration, supplemental copper at a level of 200 p.p.m. did not prevent the appearance of toxicity symptoms. Although molybdenum has been reported to increase the dietary requirement for copper (21, 24, 28, 40) it is possible that this is a result of interference with some other factor involved in copper metabolism and or reduced absorption of copper due to hypermotility of the gastrointestinal tract. Feeding high levels of molybdenum to calves resulted in a reduced liver copper concentration in three out of four animals. The value observed for the fourth animal was slightly higher than its control and may have been the result of the additional time spent on experimental rations, as well as an almost complete cessation of scouring during the latter part of the experimental period. Dick (30) has presented evidence for the existence of a factor in lucerne hay, other than copper, that is capable of preventing the appearance of toxicity symptoms in molybdenum supplemented sheep.

Adrenalectomy of both control and molybdenum supplemented young rats showed no apparent effect on liver copper concentration. This is in agreement with the findings of Koselka and Pedrero (62). The intramuscular administration of vitamin B_{12} did not have an appreciable effect on liver copper concentration. High levels of molybdenum fed to male rats over a prolonged period of time had no permanent effect on liver copper concentration as the values observed were within the normal range. Insufficient numbers of mature female rats prevented any

definite conclusions concerning the effect of molybdenum supplementation on liver copper concentration, however, there appeared to be an accumulation of copper with increased age.

A relationship between the phosphorus level in the diet and molybdenum toxicity has been observed. Rats on a low phosphorus ration were more seriously affected than were those on a normal phosphorus ration up to approximately ten weeks of age, after which there was no apparent difference between the two groups. This is in contrast to the reduced gains observed in the male control rats on the low phosphorus ration. The level of phosphorus in the diet had no effect on the copper concentration in the livers of molybdenum supplemented rats. Comar et al. (21) have observed that the simultaneous oral administration of phosphorus and molybdenum increased the absorption of molybdenum from the gastrointestinal tract. The low phosphorus ration used may have resulted in a reduced absorption of molybdenum with a correspondingly higher concentration in the gastrointestinal tract. If this were the case, it is possible that molybdenum may exert a toxic effect in the lower portion of the gastrointestinal tract of the rat.

The similarity of molybdenum toxicity symptoms with those of deficiencies of certain members of the vitamin B complex prompted a study of the relationship of molybdenum to these vitamins. The blood levels of nicotinic acid, pantothenic acid, riboflavin and vitamin B_{12} were determined for control and molybdenum supplemented calves after the appearance of toxicity symptoms. Molybdenum appeared to cause a slight reduction in the level of vitamin B_{12} but had no appreciable

effect on the other vitamins. The vitamin B_{12} levels of the livers were determined for calves in the control and molybdenum supplemented groups. One calf in the molybdenum supplemented group showed a marked depletion of vitamin B_{12} and the remainder showed lower levels than observed in the control group. Molybdenum did not appear to have an effect on the vitamin B_{12} content of rat livers, however, since rat livers contain a very low level of vitamin B_{12} and there are certain experimental difficulties little significance is attached to this finding. The results obtained with calves suggested that molybdenum may interfere with the synthesis, absorption or increase the requirement for vitamin B_{12}. The effect of molybdenum on the "folic acid conjugase" system was investigated and there was no evidence to indicate that molybdenum in the form of sodium molybdate, ammonium molybdate or molybdenum trioxide interfered with the normal functioning of this system in the rat. Urinary excretion studies on pantothenic acid and folic acid indicated that molybdenum may interfere with pantothenic acid metabolism in the rat, however, the results obtained were inconclusive.

Balance studies with calves indicated that molybdenum at a level of 200 p.p.m. had no apparent effect upon the absorption and excretion of calcium and phosphorus. Radioisotope studies with these and additional calves were undertaken primarily to ascertain the effect of molybdenum on calcium metabolism and also to study the movement in the body of molybdenum itself. High dietary levels of molybdenum had no effect on the disappearance of intravenously administered radio-

calcium from the blood which indicated no molybdenum effect on exchange into bone or the transport of radiocalcium from the blood into the other compartments. Molybdenum also had little effect on the fecal and urinary excretion of the radiocalcium.

From radiochemical and autoradiographic observation on the bones it appeared that molybdenum did not cause any change in the rate of bone growth. However, one of the molybdenum supplemented animals showed a more rapid removal of radiocalcium from the site of original laydown in the epiphysis of the long bones. This increased rate of removal was reflected by a higher level of radiocalcium in the blood and soft tissues. This altered behavior of calcium as a result of a high dietary level of molybdenum may be one of the factors responsible for the high incidence of bone fractures that have been observed (26, 28) in young cattle on molybdenum toxic pastures. High dietary molybdenum had little or no effect on the rate of disappearance of radiomolybdenum from the blood nor on the tissue accumulation and excretion.

SUMMARY

High dietary levels of molybdenum were fed to rats and calves. In general, the usual symptoms of molybdenum toxicity were observed: rats exhibited symmetrical alopecia, decreased body weight gains and an altered copper metabolism; calves exhibited scouring, decreased body weight gains, changes in hair coat condition and color and decreased liver copper levels. The following conclusions may be stated:

(1) Molybdenum induced alopecia was prevented or corrected by adrenalectomy or intramuscular injection of vitamin B_{12}.

(2) A level of molybdenum capable of producing toxicity symptoms during the period between weaning and sexual maturity in the rat had little or no effect after sexual maturity.

(3) Molybdenum was more toxic when superimposed on a low phosphorus diet than on a normal phosphorus diet.

(4) Molybdenum in the diet of the rat had no apparent effect on the utilization of a folic acid conjugate as determined by blood levels and urinary excretion of free folic acid.

(5) Molybdenum appeared to interfere with the normal metabolism of vitamin B_{12} in calves.

(6) High dietary levels of molybdenum had little or no effect on the disappearance of intravenously administered radiocalcium from the blood and on fecal and urinary excretion of the calcium.

(7) High dietary levels of molybdenum resulted in the more rapid resorption of radiocalcium from the site of original laydown in

the epiphysis of the long bones.

(8) High dietary levels of molybdenum had little or no effect on the rate of disappearance of intravenously administered radio-molybdenum from the blood nor on the tissue accumulation and excretion.

RECOMMENDATIONS FOR FUTURE RESEARCH

The results obtained in this study indicated that future research directed along the following lines would be desirable.

(1) An extension of the study on the relationship between molybdenum and certain vitamins and hormones.

(2) An investigation designed to determine whether molybdenum exerted its primary effect in the gastrointestinal tract or after absorption.

(3) An investigation to determine the interrelationships between cobalt, copper and molybdenum in the nutrition of ruminants.

(4) Further balance studies to determine the effect of molybdenum on calcium and phosphorus metabolism during different stages of molybdenum toxicity. The use of radioisotopes should facilitate these studies.

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BIOGRAPHICAL ITEMS

The writer was born July 4, 1920 in Center Junction, Iowa. He received his elementary school training in Oxford Junction, Iowa and was graduated from Oxford Junction High School in 1937.

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This dissertation was prepared under the direction of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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